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(54) Title: ALPHA-AMYLASE MUTANTS WITH ALTERED PROPERTIES

(57) Abstract: The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered stability, in particular at high temperatures and/or at low pH relative, and/or low Ca²⁺ to the parent alpha-amylase.

Alpha-amylase mutants with altered properties

FIELD OF THE INVENTION

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: stability under, e.g., high temperature and/or low pH conditions, in particular at low calcium concentrations. The variant of the invention are suitable for starch conversion, ethanol production, laundry wash, dish wash, hard surface cleaning, textile desizing, and/or sweetener production.

BACKGROUND OF THE INVENTION

Alpha-Amylases (alpha-1,4-glucan-4-glucanohydrolases, E.C. 3.2.1.1) constitute a group of enzymes, which catalyze hydrolysis of starch and other linear and branched 1,4-glucosidic oligo- and polysaccharides.

BRIEF DISCLOSURE OF THE INVENTION

The object of the present invention is to provide Termamyl-like amylases which variants in comparison to the corresponding parent alpha-amylase, i.e., un-mutated alpha-amylase, has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: stability under, e.g., high temperature and/or low pH conditions, in particular at low calcium concentrations.

Nomenclature

In the present description and claims, the conventional one-letter and three-letter codes for amino acid residues are

used. For ease of reference, alpha-amylase variants of the invention are described by use of the following nomenclature:

Original amino acid(s): position(s): substituted amino acid(s)

According to this nomenclature, for instance the substitution of alanine for asparagine in position 30 is shown as:

Ala30Asn or A30N

a deletion of alanine in the same position is shown as:

Ala30* or A30*

and insertion of an additional amino acid residue, such as lysine, is shown as:

Ala30AlaLys or A30AK

A deletion of a consecutive stretch of amino acid residues, such as amino acid residues 30-33, is indicated as (30-33)* or Δ(A30-N33).

Where a specific alpha-amylase contains a "deletion" in comparison with other alpha-amylases and an insertion is made in such a position this is indicated as:

*36Asp or *36D

for insertion of an aspartic acid in position 36.

Multiple mutations are separated by plus signs, i.e.:

Ala30Asp + Glu34Ser or A30N+E34S

representing mutations in positions 30 and 34 substituting alanine and glutamic acid for asparagine and serine, respectively.

When one or more alternative amino acid residues may be inserted in a given position it is indicated as

A30N,E or

A30N or A30E

Furthermore, when a position suitable for modification is identified herein without any specific modification being suggested, it is to be understood that any amino acid residue may be substituted for the amino acid residue present in the

position. Thus, for instance, when a modification of an alanine in position 30 is mentioned, but not specified, it is to be understood that the alanine may be deleted or substituted for any other amino acid, i.e., any one of:

5 R,N,D,A,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V.

Further, "A30X" means any one of the following substitutions:

A30R, A30N, A30D, A30C, A30Q, A30E, A30G, A30H, A30I, A30L, A30K, A30M, A30F, A30P, A30S, A30T, A30W, A30Y, or A30 V; or in short: A30R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V.

10 If the parent enzyme - used for the numbering - already has the amino acid residue in question suggested for substitution in that position the following nomenclature is used:

"X30N" or "X30N,V" in the case where for instance one or N or V is present in the wildtype.

15 Thus, it means that other corresponding parent enzymes are substituted to an "Asn" or "Val" in position 30.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an alignment of the amino acid sequences of
20 five parent Termamyl-like alpha-amylases. The numbers on the extreme left designate the respective amino acid sequences as follows:

1: SEQ ID NO: 4 (SP722)

2: SEQ ID NO: 2 (SP690)

25 3: SEQ ID NO: 10 (BAN)

4: SEQ ID NO: 8 (BLA)

5: SEQ ID NO: 6 (BSG).

DETAILED DISCLOSURE OF THE INVENTION

30 The object of the present invention is to provide Termamyl-like amylases, which variants have alpha-amylase activity and exhibits altered stability at high temperatures and/or at low pH, in particular at low calcium concentrations.

Termamyl-like alpha-amylases

A number of alpha-amylases produced by *Bacillus* spp. are highly homologous (identical) on the amino acid level.

The identity of a number of known *Bacillus* alpha-amylases can be found in the below Table 1:

Table 1

	Percent identity	707	AP137	BAN	BSG	SP690	SP722	AA560	Termamyl
			8						1
707	100.0		86.4	66.9	66.5	87.6	86.2	95.5	68.1
AP137	86.4		100.0	67.1	68.1	95.1	86.6	86.0	69.4
BAN	66.9		67.1	100.0	65.6	67.1	68.8	66.9	80.7
BSG	66.5		68.1	65.6	100.0	67.9	67.1	66.3	65.4
SP690	87.6		95.1	67.1	67.9	100.0	87.2	87.0	69.2
SP722	86.2		86.6	68.8	67.1	87.2	100.0	86.8	70.8
AA560	95.5		86.0	66.9	66.3	87.0	86.8	100.0	68.3
Termamyl	68.1		69.4	80.7	65.4	69.2	70.8	68.3	100.0

10

For instance, the *B. licheniformis* alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 8 (commercially available as Termamyl™) has been found to be about 81% homologous with the *B. amyloliquefaciens* alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 10 and about 65% homologous with the *B. stearothermophilus* alpha-amylase (BSG) comprising the amino acid sequence shown in SEQ ID NO: 6. Further homologous alpha-amylases include SP690 and SP722 disclosed in WO 95/26397 and further depicted in SEQ ID NO: 2 and SEQ ID NO: 4, respectively, herein. Other amylases are the AA560 alpha-amylase derived from *Bacillus* sp. and shown in SEQ ID NO: 12, and the #707 alpha-amylase derived from *Bacillus* sp., shown in SEQ ID NO: 13 and described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31.

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The KSM AP1378 alpha-amylase is disclosed in WO 97/00324 (from KAO Corporation).

Still further homologous alpha-amylases include the alpha-amylase produced by the *B. licheniformis* strain described in
5 EP 0252666 (ATCC 27811), and the alpha-amylases identified in WO 91/00353 and WO 94/18314. Other commercial Termamyl-like alpha-amylases are comprised in the products sold under the following tradenames: Optitherm™ and Takatherm™ (Solvay); Maxamyl™ (available from Gist-brocades/Genencor), Spezym AA™
10 and Spezyme Delta AATM (available from Genencor), and Keistase™ (available from Daiwa), Dex 10, GC 521 (available from Genencor) and Ultraphlow (from Enzyme Biosystems).

Because of the substantial homology found between these alpha-amylases, they are considered to belong to the same
15 class of alpha-amylases, namely the class of "Termamyl-like alpha-amylases".

Accordingly, in the present context, the term "Termamyl-like" alpha-amylase" is intended to indicate an alpha-amylase, in particular *Bacillus* alpha-amylase, which, at the amino acid
20 level, exhibits a substantial identity to Termamyl™, i.e., the *B. licheniformis* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8, herein.

In other words, all the following alpha-amylases, which has the amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10,
25 12 and 13 herein are considered to be "Termamyl-like alpha-amylase". Other Termamyl-like alpha-amylases are alpha-amylases i) which displays at least 60%, such as at least 70%, e.g., at least 75%, or at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% homology
30 (identity) with at least one of said amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, and 13, and/or is encoded by a DNA sequence which hybridizes to the DNA

sequences encoding the above-specified alpha-amylases which are apparent from SEQ ID NOS: 1, 3, 5, 7, 9, and of the present specification (which encoding sequences encode the amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10 and 12 herein, respectively).

Homology

The homology may be determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (described above). Thus, Gap GCGv8 may be used with the default scoring matrix for identity and the following default parameters: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, respectively for nucleic acidic sequence comparison, and GAP creation penalty of 3.0 and GAP extension penalty of 0.1, respectively, for protein sequence comparison. GAP uses the method of Needleman and Wunsch, (1970), J.Mol. Biol. 48, p.443-453, to make alignments and to calculate the identity.

A structural alignment between Termamyl (SEQ ID NO: 8) and, e.g., another alpha-amylase may be used to identify equivalent/corresponding positions in other Termamyl-like alpha-amylases. One method of obtaining said structural alignment is to use the Pile Up programme from the GCG package using default values of gap penalties, i.e., a gap creation penalty of 3.0 and gap extension penalty of 0.1. Other structural alignment methods include the hydrophobic cluster analysis (Gaboriaud et al., (1987), FEBS LETTERS 224, pp. 149-155) and reverse threading (Huber, T; Torda, AE, PROTEIN SCIENCE Vol. 7, No. 1 pp. 142-149 (1998)).

Hybridisation

The oligonucleotide probe used in the characterisation of the Termamyl-like alpha-amylase above may suitably be prepared on the basis of the full or partial nucleotide or amino acid
5 sequence of the alpha-amylase in question.

Suitable conditions for testing hybridisation involve pre-soaking in 5xSSC and prehybridizing for 1 hour at 40°C in a solution of 20% formamide, 5xDenhardt's solution, 50mM sodium phosphate, pH 6.8, and 50mg of denatured sonicated calf thymus
10 DNA, followed by hybridisation in the same solution supplemented with 100 mM ATP for 18 hours at 40°C, followed by three times washing of the filter in 2xSSC, 0.2% SDS at 40°C for 30 minutes (low stringency), preferred at 50°C (medium stringency), more preferably at 65°C (high stringency), even
15 more preferably at 75°C (very high stringency). More details about the hybridisation method can be found in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989.

In the present context, "derived from" is intended not only
20 to indicate an alpha-amylase produced or producible by a strain of the organism in question, but also an alpha-amylase encoded by a DNA sequence isolated from such strain and produced in a host organism transformed with said DNA sequence. Finally, the term is intended to indicate an alpha-amylase,
25 which is encoded by a DNA sequence of synthetic and/or cDNA origin and which has the identifying characteristics of the alpha-amylase in question. The term is also intended to indicate that the parent alpha-amylase may be a variant of a naturally occurring alpha-amylase, i.e., a variant, which is
30 the result of a modification (insertion, substitution, deletion) of one or more amino acid residues of the naturally occurring alpha-amylase.

Parent Termamyl-like Alpha-amylases

According to the invention all Termamyl-like alpha-amylases, as defined above, may be used as the parent (i.e., backbone) alpha-amylase. In a preferred embodiment of the invention the parent alpha-amylase is derived from *B. licheniformis*, e.g., one of those referred to above, such as the *B. licheniformis* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

10

Parent hybrid Termamyl-like Alpha-amylases

The parent alpha-amylase (i.e., backbone alpha-amylase) may also be a hybrid alpha-amylase, i.e., an alpha-amylase, which comprises a combination of partial amino acid sequences derived from at least two alpha-amylases.

The parent hybrid alpha-amylase may be one, which on the basis of amino acid homology (identity) and/or DNA hybridization (as defined above) can be determined to belong to the Termamyl-like alpha-amylase family. In this case, the hybrid alpha-amylase is typically composed of at least one part of a Termamyl-like alpha-amylase and part(s) of one or more other alpha-amylases selected from Termamyl-like alpha-amylases or non-Termamyl-like alpha-amylases of microbial (bacterial or fungal) and/or mammalian origin.

Thus, the parent hybrid alpha-amylase may comprise a combination of partial amino acid sequences deriving from at least two Termamyl-like alpha-amylases, or from at least one Termamyl-like and at least one non-Termamyl-like bacterial alpha-amylase, or from at least one Termamyl-like and at least one fungal alpha-amylase. The Termamyl-like alpha-amylase from which a partial amino acid sequence derives, may be any of the specific Termamyl-like alpha-amylase referred to herein.

For instance, the parent alpha-amylase may comprise a C-terminal part of an alpha-amylase derived from a strain of *B. licheniformis*, and a N-terminal part of an alpha-amylase derived from a strain of *B. amyloliquefaciens* or from a strain of *B. stearothermophilus*. For instance, the parent alpha-amylase may comprise at least 430 amino acid residues of the C-terminal part of the *B. licheniformis* alpha-amylase, and may, e.g., comprise a) an amino acid segment corresponding to the 37 N-terminal amino acid residues of the *B. amyloliquefaciens* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 10 and an amino acid segment corresponding to the 445 C-terminal amino acid residues of the *B. licheniformis* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8, or a hybrid Termamyl-like alpha-amylase being identical to the Termamyl sequence, i.e., the *Bacillus licheniformis* alpha-amylase shown in SEQ ID NO: 8, except that the N-terminal 35 amino acid residues (of the mature protein) has been replaced by the N-terminal 33 residues of BAN (mature protein), i.e., the *Bacillus amyloliquefaciens* alpha-amylase shown in SEQ ID NO: 10; or b) an amino acid segment corresponding to the 68 N-terminal amino acid residues of the *B. stearothermophilus* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 6 and an amino acid segment corresponding to the 415 C-terminal amino acid residues of the *B. licheniformis* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

Another suitable parent hybrid alpha-amylase is the one previously described in WO 96/23874 (from Novo Nordisk) constituting the N-terminus of BAN, *Bacillus amyloliquefaciens* alpha-amylase (amino acids 1-300 of the mature protein) and the C-terminus from Termamyl (amino acids 301-483 of the mature protein).

In a preferred embodiment of the invention the parent Termamyl-like alpha-amylase is a hybrid alpha-amylase of SEQ ID NO: 8 and SEQ ID NO: 10. Specifically, the parent hybrid Termamyl-like alpha-amylase may be a hybrid alpha-amylase comprising the 445 C-terminal amino acid residues of the B. licheniformis alpha-amylase shown in SEQ ID NO: 8 and the 37 N-terminal amino acid residues of the alpha-amylase derived from B. amyloliquefaciens shown in SEQ ID NO: 10, which may suitably further have the following mutations:

10 H156Y+A181T+N190F+A209V+Q264S (using the numbering in SEQ ID NO: 8). The latter mentioned hybrid is used in the examples below and is referred to as LE174.

Other specifically contemplated parent alpha-amylase include LE174 with fewer mutations, i.e., the right above mentioned hybrid having the following mutations:

15 A181T+N190F+A209V+Q264S; N190F+A209V+Q264S; A209V+Q264S; Q264S; H156Y+N190F+A209V+Q264S; H156Y+A209V+Q264S; H156Y+Q264S; H156Y+A181T+A209V+Q264S; H156Y+A181T+Q264S; H156Y+Q264S; H156Y+A181T+N190F+Q264S; H156Y+A181T+N190F; 20 H156Y+A181T+N190F+A209V. These hybrids are also considered to be part of the invention.

In a preferred embodiment the parent Termamyl-like alpha amylase is LE174, SP722, or AA560 including any of LE174+G48A+T49I+G107A+I201F; LE174+M197L; 25 LE174+G48A+T49I+G107A+M197L+I201F, or SP722+D183*+G184*; SP722+D183*+G184*+N195F; SP722+D183*+G184*+M202L; SP722+D183*+G184*+N195F+M202L; BSG+I181*+G182*; BSG+I181*+G182*+N193F; BSG+I181*+G182*+M200L; BSG+I181*+G182*+N193F+M200L; 30 AA560+D183*+G184*; AA560+D183*+G184*+N195F; AA560+D183*+G184*+M202L; AA560+D183*+G184*+N195F+M202L.

Other parent alpha-amylases contemplated include LE429, which is LE174 with an additional substitution in I201F.

According to the invention LE335 is the alpha-amylase, which in comparison to LE429 has additional substitutions in T49I+G107A; LE399 is LE335+G48A, i.e., LE174, with G48A+T49I+G107A+I201P.

5

Altered properties

The following section discusses the relationship between mutations, which are present in variants of the invention, and desirable alterations in properties (relative to those of a parent Termamyl-like alpha-amylase), which may result therefrom.

As mentioned above the invention relates to Termamyl-like alpha-amylases with altered properties (as mentioned above), in particular at high temperatures and/or at low pH, in particular at low calcium concentrations.

In the context of the present invention "high temperature" means temperatures from 70-120°C, preferably 80-100°C, especially 85-95°C.

In the context of the present invention the term "low pH" means from a pH in the range from 4-6, preferably 4.2-5.5, especially 4.5-5.

In the context of the present invention the term "high pH" means from a pH in the range from 8-11, especially 8.5-10.6.

In the context of the present invention the term "low calcium concentration" means free calcium levels lower than 60 ppm, preferably 40 ppm, more preferably 25 ppm, especially 5 ppm calcium.

Parent Termamyl-like alpha-amylase specifically contemplated in connection with going through the specifically contemplated altered properties are the above mentioned parent Termamyl-like alpha-amylase and parent hybrid Termamyl-like alpha-amylases.

The Termamyl® alpha-amylase is used as the starting point, but corresponding positions in, e.g., the SP722, ESG, BAN, AA560, SP690, KSM AP1378, and #707 should be understood as disclosed and specifically contemplated too.

5 In a preferred embodiment the variant of the invention has in particular at high temperatures and/or at low pH.

In an aspect the invention relates to variant with altered properties as mentioned above.

In the first aspect a variant of a parent Termamyl-like
10 alpha-amylase, comprising an alteration at one or more positions (using SEQ ID NO: 8 for the amino acid numbering) selected from the group of:

49, 60, 104, 132, 161, 170, 176, 179, 180, 181, 183, 200, 203,
204, 207, 212, 237, 239, 250, 280, 298, 318, 374, 385, 393,
15 402, 406, 427, 430, 440, 444, 447, 482,

wherein

(a) the alteration(s) are independently

(i) an insertion of an amino acid downstream of the amino acid which occupies the position,

20 (ii) a deletion of the amino acid which occupies the position, or

(iii) a substitution of the amino acid which occupies the position with a different amino acid,

(b) the variant has alpha-amylase activity and (c) each
25 position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.

In Termamyl® (SEQ ID NO: 8) such corresponding positions are:

30 T49; D60; N104; E132; D161; K170; K176; G179; K180; A181; D183;
D200; Y203; D204; D207; I212; K237; S239; E250; N280; Q298;
L318; Q374; E385; Q393; Y402; H406; L427 D430; V440; N444; E447;
Q482.

In SP722 (SEQ ID NO: 4) the corresponding positions are:
 T51; D62; N106; D134; D163; Q172; K179; G184; K185; A186;
 D188; D205; M208; D209; X212; L217, K242, S244, N255, N285,
 S303, M323; D387, N395; Y404; H408; I429; D432; V442; K446;
 5 Q449; K484.

Corresponding positions in other parent alpha-amylases can be found by alignment as described above and shown in the alignment in Fig. 1.

In a preferred embodiment the variant of the invention
 10 (using SEQ ID NO: 8 (Termamyl™) for the numbering) has one or more of the following substitutions:

T49I; D60N; N104D; E132A,V,P; D161N; K170Q; K176R; G179N; K180T;
 A181N; D183N; D200N; X203Y; D204S; D207V,E,L,G; X212I; K237P;
 S239W; E250G,F; N280S; X298Q; L318M; Q374R; E385V; Q393R; Y402F;
 15 H406L,W; L427I D430N; V440A; N444R,K; E447Q,K; Q482K.

In a preferred embodiment the variant of the invention (using SEQ ID NO: 4 (SP722) for the numbering) has one or more of the following substitutions:

T51I; D62N; N106D; D134A,V,P; D163N; X172Q; K179R; G184N;
 20 K185T; A186N; D188N; D205N; M208Y; D209S; X212V,E,L,G; L217I,
 K242P, S244W, N255G,F, N285S, S303Q, X323M; D387V, N395E;
 Y404F; H408L,W; X429I; D432N; V442A; X446R,K; X449Q,K; X484K,
 using SEQ ID NO: 4 (SP722) for the numbering.

Preferred double, triple and multi-mutations - using SEQ ID
 25 NO: 8 as the basis for the numbering - are selected from the group consisting of:

T49I+D60N; T49I+D60N+E132A; T49I+D60N+E132V;
 T49I+D60N+E132V+K170Q; T49I+D60N+E132A+K170Q;
 T49I+D60N+E132V+K170Q+K176R; T49I+D60N+E132A+K170Q+K176R;
 30 T49I+D60N+E132V+K170Q+K176R+D207V;
 T49I+D60N+E132A+K170Q+K176R+D207V;
 T49I+D60N+E132V+K170Q+K176R+D207E;
 T49I+D60N+E132A+K170Q+K176R+D207E;

- T49I+D60N+E132V+K170Q+K176R+D207V+E250G;
T49I+D60N+E132A+K170Q+K176R+D207V+E250G;
T49I+D60N+E132V+K170Q+K176R+D207E+E250G;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G;
5 T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S;
T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S;
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E385V;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
E385V;
25 T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
E385V+Q393R;
T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
E385V+Q393R;
T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
30 E385V+Q393R;
T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R;

- T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
E385V+Q393R+Y402F;
- T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F;
- 5 T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F;
- T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R+Y402F;
- T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
10 E385V+Q393R+Y402F+H406L;
- T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F+H406L;
- T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F+H406L;
- 15 T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R+Y402F+H406L;
- T49I+D60N+E132V+K170Q+K176R+D207V+E250G+N280S+L318M+Q373R+
E385V+Q393R+Y402F+H406L+L427I;
- T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
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E385V+Q393R+Y402F+H406L+L427I;
- T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R+Y402F+H406L+L427I;
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- T49I+D60N+E132A+K170Q+K176R+D207V+E250G+N280S+L318M+Q374R+
E385V+Q393R+Y402F+H406L+L427I+V440A;
- T49I+D60N+E132V+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+
30 E385V+Q393R+Y402F+H406L+L427I+V440A;
- T49I+D60N+E132A+K170Q+K176R+D207E+E250G+N280S+L318M+Q374R+E385
V+ Q393R+Y402F+H406L+L427I+V440A;

- D60N+E132A; D60N+E132V; D60N+E132V+K170Q; D60N+E132A+K170Q;
D60N+E132V+K170Q+K176R; T49I+D60N+E132A+K170Q+K176R;
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T49I+D60N+E132A+K170Q+K176R+D207V;
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E385V+Q393R+Y402F+H406L+L427I;
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D430N;
H406W+D430N; N444K+E447Q+Q482K; E447Q+Q482K;
N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+

- H406W+D430N+N444R+N444K+E447K+Q482K;
 D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
 D430N+N444R+N444K+E447K+Q482K;
 N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;
 5 D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;
 H406W+D430N; N444K+E447K+Q482K; E447K+Q482K;
 N104D+D161N+A181N+D183N+D200N+D204S+K237P+S239W;
 N104D+D161N+A181N+D183N+D200N+D204S+K237P;
 N104D+D161N+A181N+D183N+D200N+D204S;
 10 D161N+A181N+D183N+D200N+D204S+K237P+S239W;
 D161N+A181N+D183N+D200N+D204S+K237P;
 D161N+A181N+D183N+D200N+D204S; K237P+S239W, using SEQ ID NO: 8
 for the numbering.

- In a preferred embodiment the variant has the following
 15 substitutions: K170Q+D207V+N280S; E132A+D207V;
 D207E+E250G+H406L+L427I; D207V+L318M; D60N+D207V+L318M;
 T49I+E132V+V440A; T49I+K176R+D207V+Y402F; Q374R+E385V+Q393R;
 N190F+A209V+Q264S; G48A+T49I+G107A+I201F; T49I+G107A+I201F;
 G48A+T49I+I201F; G48A+T49I+G107A; T49I+I201F; T49I+G107A;
 20 G48A+T49I;
 D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
 D430N+N444K+E447Q+Q482K using SEQ ID NO: 8 for the numbering.
 Specific variant include: LE399; LE174+G48A+T49I+G107A;
 LE174+G48A+T49I+I201F; LE174+G48A+G107A+I201F;
 25 LE174+T49I+G107A+I201F; LE174+G48A+T49I; LE174+G48A;
 LE174+G107A+I201F; LE174+I201F, are specifically contemplated
 variants of the invention.

Stability

- 30 In the context of the present invention, mutations
 (including amino acid substitutions and deletion) of
 importance with respect to achieving altered stability, in
 particular improved stability (i.e., higher or lower), at

especially high temperatures (i.e., 70-120°C) and/or extreme pH (i.e. low or high pH, i.e, pH 4-6 or pH 8-11, respectively), in particular at free (i.e., unbound, therefore in solution) calcium concentrations below 60 ppm, include any
5 of the mutations listed in the "Altered properties" section. The stability may be determined as described in the "Materials & Methods" section below.

General mutations in variants of the invention

10 A variant of the invention may in one embodiment comprise one or more modifications in addition to those outlined above. Thus, it may be advantageous that one or more Proline (Pro) residues present in the part of the alpha-amylase variant which is modified is/are replaced with a non-Proline residue
15 which may be any of the possible, naturally occurring non-Proline residues, and which preferably is an Alanine, Glycine, Serine, Threonine, Valine or Leucine.

Analogously, in one embodiment one or more Cysteine residues present in the parent alpha-amylase may be replaced
20 with a non-Cysteine residue such as Serine, Alanine, Threonine, Glycine, Valine or Leucine.

Furthermore, a variant of the invention may - either as the only modification or in combination with any of the above outlined modifications - be modified so that one or more Asp
25 and/or Glu present in an amino acid fragment corresponding to the amino acid fragment 185-209 of SEQ ID NO: 10 is replaced by an Asn and/or Gln, respectively. Also of interest is the replacement, in the Termamyl-like alpha-amylase, of one or more of the Lys residues present in an amino acid fragment
30 corresponding to the amino acid fragment 185-209 of SEQ ID NO: 10 by an Arg.

It is to be understood that the present invention encompasses variants incorporating two or more of the above outlined modifications.

Furthermore, it may be advantageous to introduce mutations
5 in one or more of the following positions (using SEQ ID NO: 8 (Termamyl) for the numbering):

M15, V128, A111, H133, W138, T149, M197, N188, A209, A210, H405, T412, in particular the following single, double or triple or multi mutations:

- 10 M15X, in particular M15T,L;
V128X, in particular V128E;
H133X, in particular H133Y;
N188X, in particular N188S,T,P;
M197X, in particular M197T,L;
- 15 A209X, in particular A209V;
M197T/W138F; M197T/W138Y; M15T/H133Y/N188S;
M15/V128E/H133Y/N188S; E119C/S130C; D124C/R127C; H133Y/T149I;
G475R, H133Y/S187D; H133Y/A209V.

20 Methods for preparing alpha-amylase variants of the invention

Several methods for introducing mutations into genes are known in the art. After a brief description of cloning of alpha-amylase-encoding DNA sequences, methods for generating mutations at specific sites within the alpha-amylase-encoding
25 sequence will be described.

Cloning a DNA sequence encoding an alpha-amylase

The DNA sequence encoding a parent alpha-amylase may be isolated from any cell or microorganism producing the alpha-
30 amylase in question, using various methods well known in the art. First, a genomic DNA and/or cDNA library should be constructed using chromosomal DNA or messenger RNA from the organism that produces the alpha-amylase to be studied. Then,

if the amino acid sequence of the alpha-amylase is known, homologous, labeled oligonucleotide probes may be synthesized and used to identify alpha-amylase-encoding clones from a genomic library prepared from the organism in question. Alternatively, a labeled oligonucleotide probe containing sequences homologous to a known alpha-amylase gene could be used as a probe to identify alpha-amylase-encoding clones, using hybridization and washing conditions of lower stringency.

Yet another method for identifying alpha-amylase-encoding clones would involve inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming alpha-amylase-negative bacteria with the resulting genomic DNA library, and then plating the transformed bacteria onto agar containing a substrate for alpha-amylase, thereby allowing clones expressing the alpha-amylase to be identified.

Alternatively, the DNA sequence encoding the enzyme may be prepared synthetically by established standard methods, e.g., the phosphoramidite method described by S.L. Beaucage and M.H. Caruthers, Tetrahedron Letters 22, 1981, pp. 1859-1869, or the method described by Matthes et al., The EMBO J. 3, 1984, pp. 801-805. In the phosphoramidite method, oligonucleotides are synthesized, e.g., in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors.

Finally, the DNA sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate, the fragments corresponding to various parts of the entire DNA sequence), in accordance with standard techniques. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., Science 239, 1988, pp. 487-491.

Site-directed mutagenesis

Once an alpha-amylase-encoding DNA sequence has been isolated, and desirable sites for mutation identified, mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites; mutant nucleotides are inserted during oligonucleotide synthesis. In a specific method, a single-stranded gap of DNA, bridging the alpha-amylase-encoding sequence, is created in a vector carrying the alpha-amylase gene. Then the synthetic nucleotide, bearing the desired mutation, is annealed to a homologous portion of the single-stranded DNA. The remaining gap is then filled in with DNA polymerase I (Klenow fragment) and the construct is ligated using T4 ligase. A specific example of this method is described in Morinaga et al. (1984). US 4,760,025 disclose the introduction of oligonucleotides encoding multiple mutations by performing minor alterations of the cassette. However, an even greater variety of mutations can be introduced at any one time by the Morinaga method, because a multitude of oligonucleotides, of various lengths, can be introduced.

Another method for introducing mutations into alpha-amylase-encoding DNA sequences is described in Nelson and Long

(1989). It involves the 3-step generation of a PCR fragment containing the desired mutation introduced by using a chemically synthesized DNA strand as one of the primers in the PCR reactions. From the PCR-generated fragment, a DNA fragment
5 carrying the mutation may be isolated by cleavage with restriction endonucleases and reinserted into an expression plasmid.

Alternative methods for providing variants of the invention include gene shuffling, e.g., as described in WO 95/22625
10 (from Affymax Technologies N.V.) or in WO 96/00343 (from Novo Nordisk A/S), or other corresponding techniques resulting in a hybrid enzyme comprising the mutation(s), e.g., substitution(s) and/or deletion(s), in question. Examples of parent alpha-amylases, which suitably may be used for
15 providing a hybrid with the desired mutations(s) according to the invention include the KSM-K36 and KSM-K38 alpha-amylases disclosed in EP 1,022,334 (hereby incorporated by reference).

Expression of alpha-amylase variants

20 According to the invention, a DNA sequence encoding the variant produced by methods described above, or by any alternative methods known in the art, can be expressed, in enzyme form, using an expression vector which typically includes control sequences encoding a promoter, operator,
25 ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

The recombinant expression vector carrying the DNA sequence encoding an alpha-amylase variant of the invention may be any vector, which may conveniently be subjected to recombinant DNA
30 procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of

which is independent of chromosomal replication, e.g., a plasmid, a bacteriophage or an extrachromosomal element, minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of *E. coli*, the *Streptomyces coelicolor* agarase gene *dagA* promoters, the promoters of the *Bacillus licheniformis* alpha-amylase gene (*amyL*), the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), the promoters of the *Bacillus amyloliquefaciens* alpha-amylase (*amyQ*), the promoters of the *Bacillus subtilis* *xylA* and *xylB* genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding *A. oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral alpha-amylase, *A. niger* acid stable alpha-amylase, *A. niger* glucoamylase, *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase.

The expression vector of the invention may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the alpha-amylase variant of the invention. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pB194, pAMB1 and pIJ702.

5 The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the *dal* genes from *B. subtilis* or *B. licheniformis*, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance. Furthermore, the vector may comprise *Aspergillus* selection markers such as *amdS*, *argB*, *niaD* and *sC*, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, e.g., as described in WO 91/17243.

15 While intracellular expression may be advantageous in some respects, e.g., when using certain bacteria as host cells, it is generally preferred that the expression is extracellular. In general, the *Bacillus* alpha-amylases mentioned herein comprise a preregion permitting secretion of the expressed protease into the culture medium. If desirable, this preregion 20 may be replaced by a different preregion or signal sequence, conveniently accomplished by substitution of the DNA sequences encoding the respective preregions.

The procedures used to ligate the DNA construct of the invention encoding an alpha-amylase variant, the promoter, 25 terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor, 1989).

30 The cell of the invention, either comprising a DNA construct or an expression vector of the invention as defined above, is advantageously used as a host cell in the

recombinant production of an alpha-amylase variant of the invention. The cell may be transformed with the DNA construct of the invention encoding the variant, conveniently by integrating the DNA construct (in one or more copies) in the host chromosome. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional methods, e.g., by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells.

The cell of the invention may be a cell of a higher organism such as a mammal or an insect, but is preferably a microbial cell, e.g., a bacterial or a fungal (including yeast) cell.

Examples of suitable bacteria are Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, or *Streptomyces lividans* or *Streptomyces murinus*, or gramnegative bacteria such as *E.coli*. The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known per se.

The yeast organism may favorably be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, e.g. *Saccharomyces cerevisiae*. The filamentous fungus may advantageously belong to a species of *Aspergillus*, e.g., *Aspergillus oryzae* or *Aspergillus niger*. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a

manner known per se. A suitable procedure for transformation of *Aspergillus* host cells is described in EP 238 023.

In a yet further aspect, the present invention relates to a method of producing an alpha-amylase variant of the invention, 5 which method comprises cultivating a host cell as described above under conditions conducive to the production of the variant and recovering the variant from the cells and/or culture medium.

The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question 10 and obtaining expression of the alpha-amylase variant of the invention. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g., as described in catalogues of the American Type Culture 15 Collection).

The alpha-amylase variant secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating 20 proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

25 Industrial Applications

The alpha-amylase variants of this invention possess valuable properties allowing for a variety of industrial applications. In particular, enzyme variants of the invention are applicable as a component in washing, dishwashing, and hard 30 surface cleaning detergent compositions.

Variant of the invention with altered properties may be used for starch processes, in particular starch conversion, especially liquefaction of starch (see, e.g., US 3,912,590, EP

patent publications Nos. 252 730 and 63 909, WO 99/19467, and WO 96/28567 all references hereby incorporated by reference). Also contemplated are compositions for starch conversion purposes, which may beside the variant of the invention also comprise a
5 AMG, pullulanase, and other alpha-amylases.

Further, variants of the invention are also particularly useful in the production of sweeteners and ethanol (see, e.g., US patent no. 5,231,017 hereby incorporated by reference), such as fuel, drinking and industrial ethanol, from starch or whole
10 grains.

A variant of the invention may also be used for textile desizing (see, e.g., WO 95/21247, US patent 4,643,736, EP 119,920 hereby incorporated by reference).

15 Detergent compositions

As mentioned above, variants of the invention may suitably be incorporated in detergent compositions. Reference is made, for example, to WO 96/23874 and WO 97/07202 for further details concerning relevant ingredients of detergent
20 compositions (such as laundry or dishwashing detergents), appropriate methods of formulating the variants in such detergent compositions, and for examples of relevant types of detergent compositions.

Detergent compositions comprising a variant of the invention
25 may additionally comprise one or more other enzymes, such as a protease, a lipase, a peroxidase, another amylolytic enzyme, glucoamylase, maltogenic amylase, CGTase and/or a cellulase, mannanase (such as Mannaway™ from Novozymes, Denmark)), pectinase, pectine lyase, cutinase, laccase, and/or another
30 alpha-amylase.

Alpha-amylase variants of the invention may be incorporated in detergents at conventionally employed concentrations. It is at present contemplated that a variant of the invention may be

incorporated in an amount corresponding to 0.00001-10 mg (calculated as pure, active enzyme protein) of alpha-amylase per liter of wash/dishwash liquor using conventional dosing levels of detergent.

Compositions

The invention also related to composition comprising a variant of the invention, and in a preferred embodiment also a *B. stearothermophilus* alpha-amylase (BSG), in particular a variant thereof.

In another embodiment the composition comprises beside a variant of the invention a glucoamylase, in particular a glucoamylase originating from *Aspergillus niger* (e.g., the G1 or G2 *A. niger* AMG disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from *Aspergillus niger* are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102, or a variant therefore, in particular a variant disclosed in WO 00/04136 or WO 01/04273 or the *Talaromyces emersonii* AMG disclosed in WO 99/28448.

A specific combination is LE399 and a variant disclosed in WO 00/04136 or WO 01/04273, in particular a variant with one or more of the following substitutions:

N9A, S56A, V59A, S119P, A246T, N313G, E342T, A393R, S394R, Y402F, E408R, in particular a variant with all mutation.

In an embodiment the composition of the invention also comprises a pullulanase, in particular a *Bacillus* pullulanase.

MATERIALS AND METHODS

Enzymes:

Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8 and also available from Novozymes.

- 5 AA560: SEQ ID NO: 12; disclosed in WO 00/60060; deposited on 25th January 1999 at DSMZ and assigned the DSMZ no. 12649. AA560 were deposited by the inventors under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure
10 at Deutsche Sammlung von Microorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig DE.

LB medium (In 1 liter H₂O: 10 g bacto-tryptone, 5 g bacto-yeast extract, 10 g NaCl, pH adjusted to 7.0 w. NaOH, autoclaved).

- 15 TY agar plates (In 1 liter H₂O: 16 g bacto-tryptone, 10 g bacto-yeast extract, 5 g NaCl, pH adjusted to 7.0 w. NaOH, and 15 g bacto-agar is added prior to autoclaving).

10% Lugol solution (Iodine/Potassium iodine solution; made by 10-fold dil. in H₂O of stock: Sigma Cat. no. L 6146).

- 20 *Bacillus subtilis* SHA273: see WO 95/10603

Plasmids

- pDN1528 contains the complete gene encoding Termamyl, amyL, the expression of which is directed by its own promoter.
25 Further, the plasmid contains the origin of replication, ori, from plasmid pUE110 and the cat gene from plasmid pC194 conferring resistance towards chloramphenicol. pDN1528 is shown in Fig. 9 of WO 96/23874.

- 30 Methods:

Low pH filter assay

Bacillus libraries are plated on a sandwich of cellulose acetate (OE 67, Schleicher & Schuell, Dassel, Germany) - and

nitrocellulose filters (Protran-Ba 85, Schleicher & Schuell, Dassel, Germany) on TY agar plates with 10 micro g/ml chloramphenicol at 37°C for at least 21 hours. The cellulose acetate layer is located on the TY agar plate.

5 Each filter sandwich is specifically marked with a needle after plating, but before incubation in order to be able to localize positive variants on the filter, and the nitrocellulose filter with bound variants is transferred to a container with citrate buffer, pH 4.5 and incubated at 80°C
10 for 20 minutes (when screening for variants in the wild type backbone) or 85°C for 60 minutes (when screening for variants in the LE399 backbone). The cellulose acetate filters with colonies are stored on the TY-plates at room temperature until use. After incubation, residual activity is detected on assay
15 plates containing 1% agarose, 0.2% starch in citrate buffer, pH 6.0. The assay plates with nitrocellulose filters are marked the same way as the filter sandwich and incubated for 2 hours at 50°C. After removal of the filters the assay plates are stained with 10% Lugol solution. Starch degrading variants
20 are detected as white spots on dark blue background and then identified on the storage plates. Positive variants are re-screened twice under the same conditions as the first screen.

Secondary screening

25 Positive transformants after rescreening are picked from the storage plate and tested in a secondary plate assay. Positive transformants are grown for 22 hours at 37°C in 5 ml LB + chloramphenicol. The Bacillus culture of each positive transformant and as a control a clone expressing the
30 corresponding backbone are incubated in citrate buffer, pH 4.5 at 90°C and samples are taken at 0, 10, 20, 30, 40, 60 and 80 minutes. A 3 micro liter sample is spotted on an assay plate.

The assay plate is stained with 10% Lugol solution. Improved variants are seen as variants with higher residual activity (detected as halos on the assay plate) than the backbone. The improved variants are determined by nucleotide sequencing.

5

Stability assay of unpurified variants:

Bacillus cultures expressing the variants to be analysed are grown for 21 hours at 37°C in 10 ml LB+chloramphenicol. 800 micro liter culture is mixed with 200 micro l citrate buffer, 10 pH 4.5. A number of 70 micro l aliquots corresponding to the number of sample time points are made in PCR tubes and incubated at 70°C (for variants in the wt backbone) or 90°C (for variants in LE399) for various time points (typically 5, 10, 15, 20, 25 and 30 minutes) in a PCR machine. The 0 min 15 sample is not incubated at high temperature. Activity in the sample is measured by transferring 20 micro l to 200 micro l of the alpha-amylase PNP-G7 substrate MPR3 ((Boehringer Mannheim Cat. no. 1660730) as described below under "Assays for Alpha-Amylase Activity". Results are plotted as percentage 20 activity (relative to the 0 time point) versus time, or stated as percentage residual activity after incubation for a certain period of time.

Fermentation and purification of alpha-amylase variants

25 A B. subtilis strain harbouring the relevant expression plasmid is streaked on a LB-agar plate with 10 micro g/ml kanamycin from -80°C stock, and grown overnight at 37°C. The colonies are transferred to 100 ml PS-1 media supplemented with 10 micro g/ml chlcamphenicol in a 500 ml shaking flask.

30

Composition of PS-1 medium:

	Pearl sugar	100 g/l
	Soy Bean Meal	40 g/l
	Na ₂ HPO ₄ , 12 H ₂ O	10 g/l
5	Fluronic TM PE 6100	0.1 g/l
	CaCO ₃	5 g/l

The culture is shaken at 37°C at 270 rpm for 5 days.

Cells and cell debris are removed from the fermentation broth by centrifugation at 4500 rpm in 20-25 minutes. Afterwards the supernatant is filtered to obtain a completely clear solution. The filtrate is concentrated and washed on a UF-filter (10000 cut off membrane) and the buffer is changed to 20mM Acetate pH 5.5. The UF-filtrate is applied on a S-sepharose F.F. and elution is carried out by step elution with 0.2M NaCl in the same buffer. The eluate is dialysed against 10mM Tris, pH 9.0 and applied on a Q-sepharose F.F. and eluted with a linear gradient from 0-0.3M NaCl over 6 column volumes. The fractions that contain the activity (measured by the Phadebas assay) are pooled, pH was adjusted to pH 7.5 and remaining color was removed by a treatment with 0.5% W/vol. active coal in 5 minutes.

Stability determination of purified variants

All stability trials of purified variants are made using the same set up. The method is as follows:

The enzyme is incubated under the relevant conditions (1-4). Samples are taken at various time points, e.g., after 0, 5, 10, 15 and 30 minutes and diluted 25 times (same dilution for all taken samples) in assay buffer (0.1M 50mM Britton buffer pH 7.3) and the activity is measured using the Phadebas assay (Pharmacia) under standard conditions pH 7.3, 37°C.

The activity measured before incubation (0 minutes) is used as reference (100%). The decline in percent is calculated as a

function of the incubation time. The table shows the residual activity after, e.g., 30 minutes of incubation.

Specific activity determination

5 The specific activity is determined using the Phadebas assay (Pharmacia) as activity/mg enzyme. The manufactures instructions are followed (see also below under "Assay for α -amylase activity").

10 Assays for Alpha-Amylase Activity

1. Phadebas assay

Alpha-amylase activity is determined by a method employing Phadebas® tablets as substrate. Phadebas tablets (Phadebas® Amylase Test, supplied by Pharmacia Diagnostic) contain a
15 cross-linked insoluble blue-colored starch polymer, which has been mixed with bovine serum albumin and a buffer substance and tabletted.

For every single measurement one tablet is suspended in a tube containing 5 ml 50 mM Britton-Robinson buffer (50 mM
20 acetic acid, 50 mM phosphoric acid, 50 mM boric acid, 0.1 mM CaCl_2 , pH adjusted to the value of interest with NaOH). The test is performed in a water bath at the temperature of interest. The alpha-amylase to be tested is diluted in x ml of 50 mM Britton-Robinson buffer. 1 ml of this alpha-amylase
25 solution is added to the 5 ml 50 mM Britton-Robinson buffer. The starch is hydrolyzed by the alpha-amylase giving soluble blue fragments. The absorbance of the resulting blue solution, measured spectrophotometrically at 620 nm, is a function of the alpha-amylase activity.

30 It is important that the measured 620 nm absorbance after 10 or 15 minutes of incubation (testing time) is in the range of 0.2 to 2.0 absorbance units at 620 nm. In this absorbance range there is linearity between activity and absorbance

(Lambert-Beer law). The dilution of the enzyme must therefore be adjusted to fit this criterion. Under a specified set of conditions (temp., pH, reaction time, buffer conditions) 1 mg of a given alpha-amylase will hydrolyze a certain amount of substrate and a blue colour will be produced. The colour intensity is measured at 620 nm. The measured absorbance is directly proportional to the specific activity (activity/mg of pure alpha-amylase protein) of the alpha-amylase in question under the given set of conditions.

10

2. Alternative method

Alpha-amylase activity is determined by a method employing the PNP-G7 substrate. PNP-G7 which is a abbreviation for p-nitrophenyl-alpha,D-maltoheptaoside is a blocked oligosaccharide which can be cleaved by an endo-amylase. Following the cleavage, the alpha-Glucosidase included in the kit digest the substrate to liberate a free PNP molecule which has a yellow colour and thus can be measured by visible spectrophotometry at $\lambda=405\text{nm}$ (400-420 nm). Kits containing PNP-G7 substrate and alpha-Glucosidase is manufactured by Boehringer-Mannheim (cat. No.1054635).

To prepare the reagent solution 10 ml of substrate/buffer solution is added to 50 ml enzyme/buffer solution as recommended by the manufacturer. The assay is performed by transferring 20 micro l sample to a 96 well microtitre plate and incubating at 25°C. 200 micro l reagent solution pre-equilibrated to 25°C is added. The solution is mixed and pre-incubated 1 minute and absorption is measured every 30 sec. over 4 minutes at OD 405 nm in an ELISA reader.

The slope of the time dependent absorption-curve is directly proportional to the activity of the alpha-amylase in question under the given set of conditions.

EXAMPLES

Example 1.

Construction, by error-prone PCR mutagenesis, of *Bacillus licheniformis* alpha-amylase variants having an improved
5 stability at low pH, high temperature and low calcium ion concentration compared to the parent enzyme.

Error-prone PCR mutagenesis and library construction

To improve the stability at low pH and low calcium
10 concentration of the parent *Bacillus licheniformis* alpha-amylase, error-prone PCR mutagenesis was performed. The plasmid pDN1528 encoding the wild-type *Bacillus licheniformis* alpha-amylase gene was utilized as template to amplify this gene with primers: 22149: 5'-CGA TTG CTG ACG CTG TTA TTT GCG-
15 3' (SEQ ID NO: 14) and 24814: 5'-GAT CAC CCG CGA TAC CGT C-3' (SEQ ID NO: 15) under PCR conditions where increased error rates leads to introduction of random point mutations. The PCR conditions utilized were: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM MgCl₂, 0.3 mM MnCl₂, 0.1mM dGTP/dATP, 0.5 mM dTTP/dCTP, and
20 2.5 units Taq polymerase per 100 micro l reaction.

The resultant PCR fragment was purified on gel and used in a PCR-based multimerization step with a gel purified vector fragment created by PCR amplification of pDN1528 with primers #24: 5'-GAA TGT ATG TCG GCC GGC AAA ACG CCG GTG A-3' (SEQ ID
25 NO: 16) and #27: 5'-GCC GCC GCT GCT GCA GAA TGA GGC AGC AAG-3' (SEQ ID NO:17) forming an overlap to the insert fragment. The multimerization reaction was subsequently introduced into *B. subtilis* (Shafikhani et al., *Biotechniques*, 23 (1997), 304-310).

30

Screening

The error-prone library described above was screened in the low pH filter assay (see "Materials & Methods"). Clones

testing positive upon rescreening was submitted to secondary screening for stability in the liquid assay described in Materials and Methods.

9 Results:

Increased stability at pH 4.5, 5 ppm calcium incubated at 90°C

Name	wt	LE488	LE489	7.19.1	8.9.1
Mutations	-	D207V	K170Q D207V N280S	E132A D207V	D207E E250G H406L L427I
Stability ¹)	-	+	+	+	+

1) A "+" indicates significant increase in stability relative to wild type.

10 Increased stability at pH 4.5, 5 ppm calcium incubated at 90°C

Name	wt	LE491	LE492	LE493	LE494	19.3.1
Mutations	-	D60N D207V L318M	T49I E132V V440A	T49I K176R D207V Y402F	Q374R E385V Q393R	N190F A209V Q264S
Stability ¹)	-	+	+	+	+	+

1) A "+" indicates significant increase in stability relative to wt.

Increased stability at pH 4.5, 5 ppm calcium incubated at 90°C

Name	wt	E132-1	D207-7	D207-6	E250-8
Mutations	-	E132P	D207L	D207G	E250F
Stability ¹)	-	+	+	+	+

15 1) A "+" indicates significant increase in stability relative to wt.

Example 2

Transfer, by site-directed mutagenesis, of a selection of mutations from Example 1 to a new (non-wild type) backbone to improve stability at low pH and low calcium ion concentration compared to the parent enzyme.

Site-directed mutagenesis

Mutations from LE493 (K176R+D207V+Y402F) were transferred to LE399 yielding LE495. This was performed by the overlap PCR method (Kirchhoff and Desrosiers, PCR Methods and Applications, 2 (1993), 301-304). 2 overlapping PCR fragments were generated by amplification of the LE399 template with the primers: Fragment A: #312 Mut176 5'-CCC GAA AGC TGA ACC GCA TCT ATA GGT TTC AAG GGA AGA CTT GGG ATT-3' (SEQ ID NO: 18) (mutated codon indicated in bold) and #290 D207overlap 5'-AGG ATG GTC ATA ATC AAA GTC GG-3' (SEQ ID NO: 19); Fragment B: #313 Mut207 5'-CCG ACT TTG ATT ATG ACC ATC CTG TTG TCG TAG CAG AGA TTA AGA GAT GGG G-3' (SEQ ID NO: 20) and #314 Mut402 5'-CGA CAA TGT CAT GGT GGT CGA AAA AAT CAT GCT GTG CTC CGT ACG-3' (SEQ ID NO: 21). Fragments A and B were mixed in equimolar ratios and subsequently the full-length fragment was amplified with the external primers: #312 Mut176 and #314 Mut402. This fragment was used in a multimerization reaction with the vector PCR fragment created with the primers #296 Y402multi 5'-TTT CGA CCA CCA TGA CAT TGT CG-3' (SEQ ID NO: 22) and #305 399Multi176 5'-TAT AGA TGC GGT TCA GCT TTC GGG-3' (SEQ ID NO: 23) on template LE399 as described above. The multimerization reaction was subsequently transformed into *E. subtilis*. Clones were screened for stability in the assay mentioned above. The presence of the mutations from LE493 in several clones with increased stability was confirmed by sequencing.

LE 497 was obtained in a similar manner by amplifying the LE399 encoding template with primers #312 Mut176 and #314 Mut402 and using the resulting PCR fragment in a multimerization reaction with a vector fragment obtained by PCR amplification of the LE399 template with the primers #296 Y402multi and #305 399Multi176.

Results:

Stabilization of LE399 variant at pH 4.5, 5ppm calcium
10 incubated at 90oC

Name	LE399	LE495	LE497
Mutations	- (backbone)	K176R D207V Y402F	K176R Y402F
Stability1)	-	+	+

1) A "+" indicates significant increase in stability relative to backbone.

CLAIMS

1. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of:
- 49, 60, 104, 132, 161, 170, 176, 179, 180, 181, 183, 200, 203, 204, 207, 212, 237, 239, 250, 280, 298, 318, 374, 385, 393, 402, 406, 427, 430, 440, 444, 447, 482,
- wherein
- (a) the alteration(s) are independently
- (i) an insertion of an amino acid downstream of the amino acid which occupies the position,
- (ii) a deletion of the amino acid which occupies the position, or
- (iii) a substitution of the amino acid which occupies the position with a different amino acid,
- (b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8.
2. The variant of claim 1, which variant has one or more of the following mutations: T49I; D60N; N104D; E132A,V,P; D161N; K170Q; K176R; G179N; K180T; A181N; D183N; D200N; X203Y; D204S; D207V,E,L,G; X212I; K237P; S239W; E250G,F; N280S; X298Q; L318M; Q374R; E385V; Q393R; Y402F; H406L,W; L427I D430N; V440A; N444R,K; E447Q,K; Q482K using SEQ ID NO: 8 for the numbering.
3. The variant of claim 1 or 2, wherein the variant has the following mutations: K170Q+D207V+N280S; E132A+D207V; D207E+E250G+H406L+L427I; D207V+L318M; D60N+D207V+L318M; T49I+E132V+V440A; T49I+K176R+D207V+Y402F; Q374R+E385V+Q393R; N190F+A209V+Q264S; G48A+T49I+G107A+I201F; T49I+G107A+I201F;

- G48A+T49I+I201F; G48A+T49I+G107A; T49I+I201F; T49I+G107A;
G48A+T49I;
- N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+
H406W+D430N+N444K+E447Q+Q482K;
- 5 D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
D430N+N444K+E447Q+Q482K;
D161N+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
D430N+N444K+E447Q+Q482K;
D161N+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
- 10 D430N+E447Q+Q482K;
N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+
H406W+D430N+E447Q+Q482K;
D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
D430N+E447Q+Q482K;
- 15 N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+
H406W+D430N;
D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
D430N;
H406W+D430N; N444K+E447Q+Q482K; E447Q+Q482K;
- 20 N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+
H406W+D430N+N444R+N444K+E447K+Q482K;
D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+
D430N+N444R+N444K+E447K+Q482K;
N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;
- 25 D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;
H406W+D430N; N444K+E447K+Q482K; E447K+Q482K;
N104D+D161N+A181N+D183N+D200N+D204S+K237P+S239W;
N104D+D161N+A181N+D183N+D200N+D204S+K237P;
N104D+D161N+A181N+D183N+D200N+D204S;
- 30 D161N+A181N+D183N+D200N+D204S+K237P+S239W;
D161N+A181N+D183N+D200N+D204S+K237P;
D161N+A181N+D183N+D200N+D204S; K237P+S239W, using SEQ ID NO: 8
for the numbering.

4. The variant of any of claims 1-3, wherein the parent Termamyl-like alpha-amylase is derived from a strain of *B. licheniformis* (SEQ ID NO: 8), *B. amyloliquefaciens* (SEQ ID NO: 10), *B. stearothermophilus* (SEQ ID NO: 6).

5. The variant of any of claims 1-4, wherein the parent Termamyl-like amylase is any of:

LE174; LE174+G48A+T49I+G107A+I201F; LE174+M197L;

10 LE174+G48A+T49I+G107A+M197L+I201F.

6. The variant of claim 1, wherein the variant is mutated in one or more of the following positions: T51I; D62N; N106D; D134A,V,P; D163N; X172Q; K179R; G184N; K185T; A186N; D188N; 15 D205N; M208Y; D209S; X212V,E,L,G; L217I, K242P, S244W, N255G,F, N285S, S303Q, X323M; D387V, N395R; Y404F; H408L,W; X429I; D432N; V442A; X446R,K; X449Q,K; X484K, using SEQ ID NO: 4 (SP722) for the numbering.

20 7. The variant of claim 1 or 6, wherein the variant has the following mutations: E212V+N285S; D134A+E212V; N255G+H408L+X429I; E212V+X323M; D62N+E212V+X323M; T51I+D134V+V442A; T51I+K179R+E212V+Y404F; D387V+N395R; N195F+X212V+K269S, when using SEQ ID NO: 4 (SP722) for the 25 numbering.

8. The variant of any of claims 1-7, wherein the parent Termamyl-like alpha-amylase is selected from the group comprising: SP690 (SEQ ID NO: 2); SP722 (SEQ ID NO: 4; AA560 30 (SEQ ID NO: 12); #707 alpha-amylase (SEQ ID NO: 13); KSM-AP1378.

9. The variant of any of claims 1-8, wherein the parent Termamyl-like amylase is any of: SP722+D183*+G184*;
SF722+D183*+G184*+N195F; SF722+D183*+G184*+M202L;
SP722+D183*+G184*+N195F+M202L; BSG+I181*+G182*;
5 BSG+I181*+G182*+N193F; BSG+I181*+G182*+M200L;
BSG+I181*+G182*+N193F+M200L;
AA560+D183*+G184*; AA560+D183*+G184*+N195F;
AA560+D183*+G184*+M202L; AA560+D183*+G184*+N195F+M202L.
- 10 10. The variant of any of claims 1-9, wherein the parent Termamyl-like alpha-amylase has an amino acid sequence which has a degree of identity to SEQ ID NO: 8 of at least 60%, preferably 70%, more preferably at least 80%, even more preferably at least about 90%, even more preferably at least 95%, even more
15 preferably at least 97%, and even more preferably at least 99%.
11. The variant of any of claims 1-10, wherein the parent Termamyl-like alpha-amylase is encoded by a nucleic acid sequence, which hybridizes under low, preferably medium,
20 preferred high stringency conditions, with the nucleic acid sequence of SEQ ID NO: 7.
12. The variant of any of claims 1-11, which variant has altered stability, in particular at high temperatures from 70-
25 120°C and/or low pH in the range from pH 4-6
13. A DNA construct comprising a DNA sequence encoding an alpha-amylase variant according to any one of claims 1-12.
- 30 14. A recombinant expression vector which carries a DNA construct according to claim 13.

15. A cell which is transformed with a DNA construct according to claim 13 or a vector according to claim 14.

16. The cell according to claim 15, which is a microorganism,
5 preferably a bacterium or a fungus.

17. The cell according to claim 16, which cell is a gram-positive bacterium, such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus*
10 *stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus* or *Bacillus thuringiensis*.

18. A composition comprising an alpha-amylase variant of any
15 of claims 1-12.

19. The composition of claim 18, further comprising a *B. stearothermophilus* (BSG) alpha-amylase, in particular SP961, particular in a ratio of 1:10 to 10:1, preferably 1:2.

20

20. The composition of claim 18 or 19, wherein the composition further comprises a glucoamylase, pullulanase and/or a phytase.

25 21. A detergent composition comprising an alpha-amylase variant according to any of claims 1-12.

22. A detergent composition of claim 21, which additionally comprises another enzyme such as a protease, a lipase, a
30 peroxidase, another amylolytic enzyme, glucoamylase, maltogenic amylase, CGTase, mannanase, cutinase, laccase and/or a cellulase.

23. Use of an alpha-amylase variant according to any of claims 1-12 or a composition according to any of claims 18-20 for starch liquefaction.

5 24. Use of an alpha-amylase variant according to any of claims 1-12 or a composition according to claims 18-20 for ethanol production.

25. Use of an alpha-amylase variant according to any one of
10 claims 1-12 or a composition according to claims 18-20 for washing and/or dishwashing.

26. Use of an alpha-amylase variant of any one of claims 1-12
or a composition according to claims 18-20 for textile
15 desizing.

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	1				50
1	HHNGTNGTMM	QYFEWHLPND	GNHWNRLRDD	ASNLRNRGIT	AIWIPPAWKG
2	HHNGTNGTMM	QYFEWYLPND	GNHWNRLRDD	AANLKSKGIT	AVWIPPAWKG
3	...VNGTLM	QYFEWYTPND	GQHWRRLQND	AEHLSDIGIT	AVWIPPAYKG
4	..ANLNGTLM	QYFEWYMPND	GQHWRRLQND	SAYLAEHGIT	AVWIPPAYKG
5	.AAPFNGTMM	QYFEWYLPDD	GTLWTKVANE	ANNLSSLGIT	ALWLPPAYKG
	51				100
1	TSQNDVGYGA	YDLYDLGEFN	QKGTVRTKYG	TRSQLESANH	ALKNNGVQVY
2	TSQNDVGYGA	YDLYDLGEFN	QKGTVRTKYG	TRNQLQAAT	SLKNNGIQVY
3	LSQSDNGYGP	YDLYDLGEFQ	QKGTVRTKYG	TKSELQDAIG	SLHSRNVQVY
4	TSQADVGYGA	YDLYDLGEFH	QKGTVRTKYG	TKGELQSAIK	SLHSRDINNY
5	TSRSDVGYGV	YDLYDLGEFN	QKGTVRTKYG	TKAQYLQAIQ	AAHAAGMQVY
	101				150
1	GDVVMNHKGG	ADATENVLAV	EVNPNRNQOE	ISGDYTIKAW	TKFDFFPGRGN
2	GDVVMNHKGG	ADGTEIVNAV	EVNRSNRNQE	TSGEYAIEAW	TKFDFFPGRGN
3	GDVVLNHHKAG	ADATEDVTAV	EVNPNARNQE	TSEHYQIKAW	TDFRFPGRGN
4	GDVVINHHKGG	ADATEDVTAV	EVDPADNRNV	ISGEHLIKAW	THFHFPGRGS
5	ADVVPDHHKGG	ADGTEWVDAV	EVNPSDRNQE	ISGTYQIQAW	TKFDFFPGRGN
	151				200
1	TYSDFKWRWY	HFDGVDWDQS	RQFQNRYYKF	RGDGKAWDWE	VDSNGNYDY
2	NHSSPKWRWY	HFDGTDWDQS	RQLQNKYYKF	RGTCKAWDWE	VDTEGNYDY
3	TYSDFKWHWY	HFDGADWDES	RKI.SRIFKF	RGECKAWDWE	VSSNGNYDY
4	TYSDFKWHWY	HFDGTDWDES	RKL.NRIYKF	.QCKAWDWE	VSNNGNYDY
5	TYSSPKWRWY	HFDGVDWDES	RKL.SRIYKF	RGICKAWDWE	VDTEGNYDY

Fig. 1

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	201				250
1	LMYADVDMDH	PEVVNELRRW	GEWYTNTLNL	DGFRIDAVKH	IKYSPTRDWL
2	LMYADVDMDH	PEVIHELNRW	GVWYTNTLNL	DGFRIDAVKH	IKYSPTRDWL
3	LMYADVDDYDH	PDVVAETKKW	GIWYANELSL	DGFRIDAACH	IKFSFLRDWV
4	LMYADIDYDH	PDVAAEIKRW	GTWYANELQL	DGFRIDAVKH	IKFSFLRDWV
5	LMYADLDMDH	PEVVTELKNW	GKQYVNTTNI	DGFRIDAVKH	IKFSFFPDWL
	251				300
1	THVRNATGKE	MFAVAEPWKN	DLGALENYLN	KTNWNHVSVD	VPLHYNLYNA
2	THVRNTTGKP	MFAVAEPWKN	DLGAIENYLN	KTSWNHSAFD	VPLHYNLYNA
3	QAVRQATGKE	MFTVAEYWQN	NAGKLENYLN	KTSFNQSVFD	VPLHFNLOAA
4	NHVREKTGKE	MFTVAEYWQN	DLGALENYLN	KTNFNHVSVD	VPLHYQFHAA
5	SYVRSQTGKP	LFTVGEYWSY	DINKLENYIT	KTDGTMSLFD	APLHNKFYTA
	301				350
1	SNSGGNYDMA	KLLNGTVVQK	HPMHAVTFVD	NHDSQPGESL	ESFVQEWFKP
2	SNSGGYYDMR	NILNGSVVQK	HPTHAVTFVD	NHDSQPGCAL	ESFVQQWFKP
3	SSQGGGYDMR	RLLDGTVVSF	HPEKAVTFVE	NHDTQPGQSL	ESTVQTWFKP
4	STQGGGYDMR	KLLNGTVVSK	HPLKSVTFVD	NHDTQPGQSL	ESTVQTWFKP
5	SKSGGAFDMR	TLMTNTLMKD	QPTLAVTFVD	NHDETPGQAL	QSWVDPWFKP
	351				400
1	LAYALILTRE	QGYPSVIFYG	YYGIPTHS..	.VFAMKAKID	PILEARQNFA
2	LAYALVLTRE	QGYPSVIFYG	YYGIPTHG..	.VPAMKSKID	PLLQARQTFA
3	LAYAFILTRE	SGYPQVIFYG	MYGKTGTSFK	EIPSLKDNIE	PILKARKEYA
4	LAYAFILTRE	SGYPQVIFYG	MYGKTGDSQR	EIPALKHKIE	PILKARKQYA
5	LAYAFILTRQ	EGYPCVIFYG	YYGIPQYN..	.IPSLKSKID	PLLIARRDYA
	401				450
1	YGTQHDYFDH	HNIIGWTREG	NTHPNNSGLA	TIMSDGPGGE	KWMYVGCNKA
2	YGTQHDYFDH	HDIIGWTREG	NSSHPNNSGLA	TIMSDGPGCN	KWMYVCKNKA
3	YGPQHDYIDH	PDVIGWTREG	DSSAAKSGLA	ALITDGPGGS	KRMYAGLKNA
4	YGAQHDYFDH	HDIIGWTREG	DSSVANSGLA	ALITDGPGGA	KRMYVGRQNA
5	YGTQHDYLDH	SDIIGWTREG	GTEKPGSGLA	ALITDGPGGS	KWMYVCKQHA

Fig. 1 (continued)

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      451                                     500
1  GQVWHDITGN KPGTVFINAD GWANFSVNGG SVSIWVKR.. .....
2  GQVWRDITGN RTGTVTINAD GWGNFSVNGG SVSVWVKQ.. .....
3  GETWYDITGN RSDTVKIGSD GWGEFHVNDG SVSIYVQ... .....
4  GETWHDITGN RSEPVVINSE GWGEFHVNGG SVSIYVQR.. .....
5  GKVPFYDLTGN RSDTVTINSD GWGEFKVNGG SVSVWVPRKT TVSTIARPIT

      501                                     519
1  .....
2  .....
3  .....
4  .....
5  TRPWTGEFVR WTEPRLVAW
```

Fig. 1 (continued)

SEQUENCE LISTING

SEQUENCE LISTING

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<220>

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His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr	
1 5 10 15	
ttg cca aat gac ggg aat cat tgg aac agg ttg agg gat gac gca gct	96
Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ala	
20 25 30	
aac tta aag agt aaa ggg ata aca gct gta tgg atc cca cct gca tgg	144
Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp	
35 40 45	
aag ggg act tcc cag aat gat gta ggt tat gga gcc tat gat tta tat	192
Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr	
50 55 60	
gat ctt gga gag ttt aac cag aag ggg acg gtt cgt aca aaa tat gga	240
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly	
65 70 75 80	
aca cgc aac cag cta cag gct gcg gtg acc tct tta aaa aat aac ggc	288
Thr Arg Asn Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly	
85 90 95	
att cag gta tat ggt gat gtc gtc atg aat cat aaa ggt gga gca gat	336
Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp	
100 105 110	
ggt acg gaa att gta aat gcg gta gaa gtg aat cgg agc aac cga aac	384
Gly Thr Glu Ile Val Asn Ala Val Glu Val Asn Arg Ser Asn Arg Asn	
115 120 125	
cag gaa acc tca gga gag tat gca ata gaa gcg tgg aca aag ttt gat	432
Gln Glu Thr Ser Gly Glu Tyr Ala Ile Glu Ala Trp Thr Lys Phe Asp	
130 135 140	
ttt cct gga aga gga aat aac cat tcc agc ttt aag tgg cgc tgg tat	480
Phe Pro Gly Arg Gly Asn Asn His Ser Ser Phe Lys Trp Arg Trp Tyr	
145 150 155 160	
cat ttt gat ggg aca gat tgg gat cag tca cgc cag ctt caa aac aaa	528
His Phe Asp Gly Thr Asp Trp Asp Gln Ser Arg Gln Leu Gln Asn Lys	
165 170 175	
ata tat aaa ttc agg gga aca ggc aag gcc tgg gac tgg gaa gtc gat	576

SEQUENCE LISTING

Ile Tyr Lys Phe Arg Gly Thr Gly	Lys Ala Trp Asp Trp Glu Val Asp	
180	185	190
aca gag aat ggc aac tat gac tat	ctt atg tat gca gac gtg gat atg	624
Thr Glu Asn Gly Asn Tyr Asp Tyr	Leu Met Tyr Ala Asp Val Asp Met	
195	200	205
gat cac cca gaa gta ata cat gaa ctt aga aac tgg gga gtg tgg tat		672
Asp His Pro Glu Val Ile His Glu Leu Arg Asn Trp Gly Val Trp Tyr		
210	215	220
acg aat aca ctg aac ctt gat gga ttt aga ata gat gca gtg aaa cat		720
Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His		
225	230	235
ata aaa tat agc ttt acg aga gat tgg ctt aca cat gtg cgt aac acc		768
Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Thr		
245	250	255
aca ggt aaa cca atg ttt gca gtg gct gag ttt tgg aaa aat gac ctt		816
Thr Gly Lys Pro Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu		
260	265	270
ggt gca att gaa aac tat ttg aat aaa aca agt tgg aat cac tct gtg		864
Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Ser Trp Asn His Ser Val		
275	280	285
ttt gat gtt cct ctc cac tat aat ttg tac aat gca tct aat agc ggt		912
Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly		
290	295	300
ggt tat tat gat atg aga aat att tta aat ggt tct gtg gtg caa aaa		960
Gly Tyr Tyr Asp Met Arg Asn Ile Leu Asn Gly Ser Val Val Gln Lys		
305	310	315
cat cca aca cat gcc gtt act ttt gtt gat aac cat gat tct cag ccc		1008
His Pro Thr His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro		
325	330	335
ggg gaa gca ttg gaa tcc ttt gtt caa caa tgg ttt aaa cca ctt gca		1056
Gly Glu Ala Leu Glu Ser Phe Val Gln Gln Trp Phe Lys Pro Leu Ala		
340	345	350
tat gca ttg gtt ctg aca agg gaa caa ggt tat cct tcc gta ttt tat		1104
Tyr Ala Leu Val Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr		
355	360	365
ggg gat tac tac ggt atc cca acc cat ggt gtt ccg gct atg aaa tct		1152
Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser		
370	375	380
aaa ata gac cct ctt ctg cag gca cgt caa act ttt gcc tat ggt acg		1200
Lys Ile Asp Pro Leu Leu Gln Ala Arg Gln Thr Phe Ala Tyr Gly Thr		
385	390	400
cag cat gat tac ttt gat cat cat gat att atc ggt tgg aca aga gag		1248
Gln His Asp Tyr Phe Asp His His Asp Ile Ile Gly Trp Thr Arg Glu		
405	410	415
gga aat agc tcc cat cca aat tca ggc ctt gcc acc att atg tca gat		1296
Gly Asn Ser Ser His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp		
420	425	430
ggt cca ggt ggt aac aaa tgg atg tat gtg ggg aaa aat aaa gcg gga		1344
Gly Pro Gly Gly Asn Lys Trp Met Tyr Val Gly Lys Asn Lys Ala Gly		
435	440	445
caa gtt tgg aga gat att acc gga aat agg aca ggc acc gtc aca att		1392

SEQUENCE LISTING

Gln Val Trp Arg Asp Ile Thr Gly Asn Arg Thr Gly Thr Val Thr Ile
 450 455 460

aat gca gac gga tgg ggt aat ttc tct gtt aat gga ggg tcc gtt tcc 1440
 Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480

gtt tgg gtg aag caa 1455
 Val Trp Val Lys Gln
 485

<210> 2
 <211> 485
 <212> PRT
 <213> Bacillus sp.

<400> 2
 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
 1 5 10 15

Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ala
 20 25 30

Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp
 35 40 45

Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
 50 55 60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
 65 70 75 80

Thr Arg Asn Gln Leu Gln Ala Ala Val Thr Ser Leu Lys Asn Asn Gly
 85 90 95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
 100 105 110

Gly Thr Glu Ile Val Asn Ala Val Glu Val Asn Arg Ser Asn Arg Asn
 115 120 125

Gln Glu Thr Ser Gly Glu Tyr Ala Ile Glu Ala Trp Thr Lys Phe Asp
 130 135 140

Phe Pro Gly Arg Gly Asn Asn His Ser Ser Phe Lys Trp Arg Trp Tyr
 145 150 155 160

His Phe Asp Gly Thr Asp Trp Asp Gln Ser Arg Gln Leu Gln Asn Lys
 165 170 175

Ile Tyr Lys Phe Arg Gly Thr Gly Lys Ala Trp Asp Trp Glu Val Asp
 180 185 190

Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met
 195 200 205

Asp His Pro Glu Val Ile His Glu Leu Arg Asn Trp Gly Val Trp Tyr
 210 215 220

Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
 225 230 235 240

Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Thr
 245 250 255

Thr Gly Lys Pro Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
 260 265 270

SEQUENCE LISTING

Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Ser Trp Asn His Ser Val
 275 280 285

Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly
 290 295 300

Gly Tyr Tyr Asp Met Arg Asn Ile Leu Asn Gly Ser Val Val Gln Lys
 305 310 315 320

His Pro Thr His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
 325 330 335

Gly Glu Ala Leu Glu Ser Phe Val Gln Gln Trp Phe Lys Pro Leu Ala
 340 345 350

Tyr Ala Leu Val Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
 355 360 365

Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
 370 375 380

Lys Ile Asp Pro Leu Leu Gln Ala Arg Gln Thr Phe Ala Tyr Gly Thr
 385 390 395 400

Gln His Asp Tyr Phe Asp His His Asp Ile Ile Gly Trp Thr Arg Glu
 405 410 415

Gly Asn Ser Ser His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430

Gly Pro Gly Gly Asn Lys Trp Met Tyr Val Gly Lys Asn Lys Ala Gly
 435 440 445

Gln Val Trp Arg Asp Ile Thr Gly Asn Arg Thr Gly Thr Val Thr Ile
 450 455 460

Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480

Val Trp Val Lys Gln
 485

<210> 3
 <211> 1455
 <212> DNA
 <213> Bacillus sp.

<220>
 <221> CDS
 <222> (1)..(1455)
 <223> SP722

<400> 3
 cat cat aat ggg aca aat ggg acg atg atg caa tac ttt gaa tgg cac 48
 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His
 1 5 10 15

ttg cct aat gat ggg aat cac tgg aat aga tta aga gat gat gct agt 96
 Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser
 20 25 30

aat cta aga aat aga ggt ata acc gct att tgg att ccg cct gcc tgg 144
 Asn Leu Arg Asn Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp
 35 40 45

SEQUENCE LISTING

aaa ggg act tgc caa aat gat gtg ggg tat gga gcc tat gat ctt tat	192
Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr	
50 55 60	
gat tta ggg gaa ttt aat caa aag ggg acg gtt cgt act aag tat ggg	240
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly	
65 70 75 80	
aca cgt agt caa ttg gag tct gcc atc cat gct tta aag aat aat ggc	288
Thr Arg Ser Gln Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly	
85 90 95	
gtt caa gtt tat ggg gat gta gtg atg aac cat aaa gga gga gct gat	336
Val Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp	
100 105 110	
gct aca gaa aac gtt ctt gct gtc gag gtg aat cca aat aac cgg aat	384
Ala Thr Glu Asn Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn	
115 120 125	
caa gaa ata tct ggg gac tac aca att gag gct tgg act aag ttt gat	432
Gln Glu Ile Ser Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp	
130 135 140	
ttt cca ggg agg ggt aat aca tac tca gac ttt aaa tgg cgt tgg tat	480
Phe Pro Gly Arg Gly Asn Thr Tyr Ser Asp Phe Lys Trp Arg Trp Tyr	
145 150 155 160	
cat ttc gat ggt gta gat tgg gat caa tca cga caa ttc caa aat cgt	528
His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg	
165 170 175	
atc tac aaa ttc cga ggt gat ggt aag gca tgg gat tgg gaa gta gat	576
Ile Tyr Lys Phe Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp	
180 185 190	
tgc gaa aat gga aat tat gat tat tta atg tat gca gat gta gat atg	624
Ser Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met	
195 200 205	
gat cat ccg gag gta gta aat gag ctt aga aga tgg gga gaa tgg tat	672
Asp His Pro Glu Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr	
210 215 220	
aca aat aca tta aat ctt gat gga ttt agg atc gat gcg gtg aag cat	720
Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His	
225 230 235 240	
att aaa tat agc ttt aca cgt gat tgg ttg acc cat gta aga aac gca	768
Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala	
245 250 255	
acg gga aaa gaa atg ttt gct gtt gct gaa ttt tgg aaa aat gat tta	816
Thr Gly Lys Glu Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu	
260 265 270	
ggt gcc ttg gag aac tat tta aat aaa aca aac tgg aat cat tct gtc	864
Gly Ala Leu Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val	
275 280 285	
ttt gat gtc ccc ctt cat tat aat ctt tat aac gcg tca aat agt gga	912
Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly	
290 295 300	
ggc aac tat gac atg gca aaa ctt ctt aat gga acg gtt gtt caa aag	960
Gly Asn Tyr Asp Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys	
305 310 315 320	

SEQUENCE LISTING

cat cca atg cat gcc gta act ttt gtg gat aat cac gat tct caa cct 1008
 His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
 325 330 335

ggg gaa tca tta gaa tca ttt gta caa gaa tgg ttt aag cca ctt gct 1056
 Gly Glu Ser Leu Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala
 340 345 350

tat gcg ctt att tta aca aga gaa caa ggc tat ccc tct gtc ttc tat 1104
 Tyr Ala Leu Ile Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
 355 360 365

ggg gac tac tat gga att cca aca cat agt gtc cca gca atg aaa gcc 1152
 Gly Asp Tyr Tyr Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala
 370 375 380

aag att gat cca atc tta gag gcg cgt caa aat ttt gca tat gga aca 1200
 Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr
 385 390 395 400

caa cat gat tat ttt gac cat cat aat ata atc gga tgg aca cgt gaa 1248
 Gln His Asp Tyr Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
 405 410 415

gga aat acc acg cat ccc aat tca gga ctt gcg act atc atg tcg gat 1296
 Gly Asn Thr Thr His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430

ggg cca ggg gga gag aaa tgg atg tac gta ggg caa aat aaa gca ggt 1344
 Gly Pro Gly Gly Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly
 435 440 445

caa gtt tgg cat gac ata act gga aat aaa cca gga aca gtt acg atc 1392
 Gln Val Trp His Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile
 450 455 460

aat gca gat gga tgg gct aat ttt tca gta aat gga gga tct gtt tcc 1440
 Asn Ala Asp Gly Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480

att tgg gtg aaa cga 1455
 Ile Trp Val Lys Arg
 485

<210> 4
 <211> 485
 <212> PRT
 <213> Bacillus sp.

<400> 4
 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His
 1 5 10 15
 Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser
 20 25 30
 Asn Leu Arg Asn Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp
 35 40 45
 Lys Gly Thr Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
 50 55 60
 Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly
 65 70 75 80
 Thr Arg Ser Gln Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly
 85 90 95

SEQUENCE LISTING

Val Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
 100 105 110
 Ala Thr Glu Asn Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn
 115 120 125
 Gln Glu Ile Ser Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
 130 135 140
 Phe Pro Gly Arg Gly Asn Thr Tyr Ser Asp Phe Lys Trp Arg Trp Tyr
 145 150 155 160
 His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg
 165 170 175
 Ile Tyr Lys Phe Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp
 180 185 190
 Ser Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met
 195 200 205
 Asp His Pro Glu Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr
 210 215 220
 Thr Asn Thr Leu Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
 225 230 235 240
 Ile Lys Tyr Ser Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala
 245 250 255
 Thr Gly Lys Glu Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
 260 265 270
 Gly Ala Leu Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
 275 280 285
 Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly
 290 295 300
 Gly Asn Tyr Asp Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys
 305 310 315 320
 His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
 325 330 335
 Gly Glu Ser Leu Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala
 340 345 350
 Tyr Ala Leu Ile Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
 355 360 365
 Gly Asp Tyr Tyr Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala
 370 375 380
 Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr
 385 390 395 400
 Gln His Asp Tyr Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
 405 410 415
 Gly Asn Thr Thr His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430
 Gly Pro Gly Gly Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly
 435 440 445
 Gln Val Trp His Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile

SEQUENCE LISTING

450	455	460
Asn Ala Asp Gly Trp	Ala Asn Phe Ser Val	Asn Gly Gly Ser Val Ser
465	470	475 480
Ile Trp Val Lys Arg		
	485	
<210> 5		
<211> 1548		
<212> DNA		
<213> Bacillus stearothermophilus		
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<222> (1)..(1548)		
<223> BSG		
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Ala Ala Pro Phe Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu		
1 5 10 15		
ccg gat gat ggc acg tta tgg acc aaa gtg gcc aat gaa gcc aac aac		96
Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn		
20 25 30		
tta tcc agc ctt ggc atc acc gct ctt tgg ctg ccg ccc gct tac aaa		144
Leu Ser Ser Leu Gly Ile Thr Ala Leu Trp Leu Pro Pro Ala Tyr Lys		
35 40 45		
gga aca agc cgc agc gac gta ggg tac gga gta tac gac ttg tat gac		192
Gly Thr Ser Arg Ser Asp Val Gly Tyr Gly Val Tyr Asp Leu Tyr Asp		
50 55 60		
ctc ggc gaa ttc aat caa aaa ggg acc gtc cgc aca aaa tac gga aca		240
Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr		
65 70 75 80		
aaa gct caa tat ctt caa gcc att caa gcc gcc cac gcc gct gga atg		288
Lys Ala Gln Tyr Leu Gln Ala Ile Gln Ala Ala His Ala Ala Gly Met		
85 90 95		
caa gtg tac gcc gat gtc gtg ttc gac cat aaa ggc ggc gct gac ggc		336
Gln Val Tyr Ala Asp Val Val Phe Asp His Lys Gly Gly Ala Asp Gly		
100 105 110		
acg gaa tgg gtg gac gcc gtc gaa gtc aat ccg tcc gac cgc aac caa		384
Thr Glu Trp Val Asp Ala Val Glu Val Asn Pro Ser Asp Arg Asn Gln		
115 120 125		
gaa atc tcc ggc acc tat caa atc caa gca tgg acg aaa ttt gat ttt		432
Glu Ile Ser Gly Thr Tyr Gln Ile Gln Ala Trp Thr Lys Phe Asp Phe		
130 135 140		
ccc ggg cgg ggc aac acc tac tcc agc ttt aag tgg cgc tgg tac cat		480
Pro Gly Arg Gly Asn Thr Tyr Ser Ser Phe Lys Trp Arg Trp Tyr His		
145 150 155 160		
ttt gac ggc gtt gat tgg gac gaa agc cga aaa ttg agc cgc att tac		528
Phe Asp Gly Val Asp Trp Asp Glu Ser Arg Arg Lys Leu Ser Arg Ile Tyr		
165 170 175		
aaa ttc cgc ggc atc ggc aaa gcg tgg gat tgg gaa gta gac acg gaa		576
Lys Phe Arg Gly Ile Gly Lys Ala Trp Asp Trp Glu Val Asp Thr Glu		
180 185 190		

SEQUENCE LISTING

aac gga aac tat gac tac tta atg tat gcc gac ctt gat atg gat cat	624
Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Leu Asp Met Asp His	
195 200 205	
ccc gaa gtc gtg acc gag ctg aaa aac tgg ggg aaa tgg tat gtc aac	672
Pro Glu Val Val Thr Glu Leu Lys Asn Trp Gly Lys Trp Tyr Val Asn	
210 215 220	
aca acg aac att gat ggg ttc cgg ctt gat gcc gtc aag cat att aag	720
Thr Thr Asn Ile Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys	
225 230 235 240	
ttc agt ttt ttt cct gat tgg ttg tcg tat gtg cgt tct cag act ggc	768
Phe Ser Phe Phe Pro Asp Trp Leu Ser Tyr Val Arg Ser Gln Thr Gly	
245 250 255	
aag ccg cta ttt acc gtc ggg gaa tat tgg agc tat gac atc aac aag	816
Lys Pro Leu Phe Thr Val Gly Glu Tyr Trp Ser Tyr Asp Ile Asn Lys	
260 265 270	
ttg cac aat tac att acg aaa aca gac gga acg atg tct ttg ttt gat	864
Leu His Asn Tyr Ile Thr Lys Thr Asp Gly Thr Met Ser Leu Phe Asp	
275 280 285	
gcc ccg tta cac aac aaa ttt tat acc gct tcc aaa tca ggg ggc gca	912
Ala Pro Leu His Asn Lys Phe Tyr Thr Ala Ser Lys Ser Gly Gly Ala	
290 295 300	
ttt gat atg cgc acg tta atg acc aat act ctc atg aaa gat caa ccg	960
Phe Asp Met Arg Thr Leu Met Thr Asn Thr Leu Met Lys Asp Gln Pro	
305 310 315 320	
aca ttg gcc gtc acc ttc gtt gat aat cat gac acc gaa ccc ggc caa	1008
Thr Leu Ala Val Thr Phe Val Asp Asn His Asp Thr Glu Pro Gly Gln	
325 330 335	
gcg ctg cag tca tgg gtc gac cca tgg ttc aaa ccg ttg gct tac gcc	1056
Ala Leu Gln Ser Trp Val Asp Pro Trp Phe Lys Pro Leu Ala Tyr Ala	
340 345 350	
ttt att cta act cgg cag gaa gga tac ccg tgc gtc ttt tat ggt gac	1104
Phe Ile Leu Thr Arg Gln Glu Gly Tyr Pro Cys Val Phe Tyr Gly Asp	
355 360 365	
tat tat ggc att cca caa tat aac att cct tcg ctg aaa agc aaa atc	1152
Tyr Tyr Gly Ile Pro Gln Tyr Asn Ile Pro Ser Leu Lys Ser Lys Ile	
370 375 380	
gat ccg ctc ctc atc gcg cgc agg gat tat gct tac gga acg caa cat	1200
Asp Pro Leu Leu Ile Ala Arg Arg Asp Tyr Ala Tyr Gly Thr Gln His	
385 390 395 400	
gat tat ctt gat cac tcc gac atc atc ggg tgg aca agg gaa ggg ggc	1248
Asp Tyr Leu Asp His Ser Asp Ile Ile Gly Trp Thr Arg Glu Gly Gly	
405 410 415	
act gaa aaa cca gga tcc gga ctg gcc gca ctg atc acc gat ggg ccg	1296
Thr Glu Lys Pro Gly Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro	
420 425 430	
gga gga agc aaa tgg atg tac gtt ggc aaa caa cac gct gga aaa gtg	1344
Gly Gly Ser Lys Trp Met Tyr Val Gly Lys Gln His Ala Gly Lys Val	
435 440 445	
ttc tat gac ctt acc ggc aac cgg agt gac acc gtc acc atc aac agt	1392
Phe Tyr Asp Leu Thr Gly Asn Arg Ser Asp Thr Val Thr Ile Asn Ser	
450 455 460	

SEQUENCE LISTING

gat gga tgg ggg gaa ttc aaa gtc aat ggc ggt tgc gtt tgc gtt tgg 1440
 Asp Gly Trp Gly Glu Phe Lys Val Asn Gly Gly Ser Val Ser Val Trp
 465 470 475 480

gtt cct aga aaa acg acc gtt tct acc atc gct cgg ccg atc aca acc 1488
 Val Pro Arg Lys Thr Thr Val Ser Thr Ile Ala Arg Pro Ile Thr Thr
 485 490 495

cga ccg tgg act ggt gaa ttc gtc cgt tgg acc gaa cca cgg ttg gtg 1536
 Arg Pro Trp Thr Gly Glu Phe Val Arg Trp Thr Glu Pro Arg Leu Val
 500 505 510

gca tgg cct tga 1548
 Ala Trp Pro
 515

<210> 6
 <211> 515
 <212> PRT
 <213> *Bacillus stearothermophilus*

<400> 6
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Pro Asp Asp Gly Thr Leu Trp Thr Lys Val Ala Asn Glu Ala Asn Asn
 20 25 30

Leu Ser Ser Leu Gly Ile Thr Ala Leu Trp Leu Pro Pro Ala Tyr Lys
 35 40 45

Gly Thr Ser Arg Ser Asp Val Gly Tyr Gly Val Tyr Asp Leu Tyr Asp
 50 55 60

Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr
 65 70 75 80

Lys Ala Gln Tyr Leu Gln Ala Ile Gln Ala Ala His Ala Ala Gly Met
 85 90 95

Gln Val Tyr Ala Asp Val Val Phe Asp His Lys Gly Gly Ala Asp Gly
 100 105 110

Thr Glu Trp Val Asp Ala Val Glu Val Asn Pro Ser Asp Arg Asn Gln
 115 120 125

Glu Ile Ser Gly Thr Tyr Gln Ile Gln Ala Trp Thr Lys Phe Asp Phe
 130 135 140

Pro Gly Arg Gly Asn Thr Tyr Ser Ser Phe Lys Trp Arg Trp Tyr His
 145 150 155 160

Phe Asp Gly Val Asp Trp Asp Glu Ser Arg Lys Leu Ser Arg Ile Tyr
 165 170 175

Lys Phe Arg Gly Ile Gly Lys Ala Trp Asp Trp Glu Val Asp Thr Glu
 180 185 190

Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Leu Asp Met Asp His
 195 200 205

Pro Glu Val Val Thr Glu Leu Lys Asn Trp Gly Lys Trp Tyr Val Asn
 210 215 220

Thr Thr Asn Ile Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys
 225 230 235 240

SEQUENCE LISTING

Phe Ser Phe Phe Pro Asp Trp Leu Ser Tyr Val Arg Ser Gln Thr Gly
 245 250 255
 Lys Pro Leu Phe Thr Val Gly Glu Tyr Trp Ser Tyr Asp Ile Asn Lys
 260 265 270
 Leu His Asn Tyr Ile Thr Lys Thr Asp Gly Thr Met Ser Leu Phe Asp
 275 280 285
 Ala Pro Leu His Asn Lys Phe Tyr Thr Ala Ser Lys Ser Gly Gly Ala
 290 295 300
 Phe Asp Met Arg Thr Leu Met Thr Asn Thr Leu Met Lys Asp Gln Pro
 305 310 315 320
 Thr Leu Ala Val Thr Phe Val Asp Asn His Asp Thr Glu Pro Gly Gln
 325 330 335
 Ala Leu Gln Ser Trp Val Asp Pro Trp Phe Lys Pro Leu Ala Tyr Ala
 340 345 350
 Phe Ile Leu Thr Arg Gln Glu Gly Tyr Pro Cys Val Phe Tyr Gly Asp
 355 360 365
 Tyr Tyr Gly Ile Pro Gln Tyr Asn Ile Pro Ser Leu Lys Ser Lys Ile
 370 375 380
 Asp Pro Leu Leu Ile Ala Arg Arg Asp Tyr Ala Tyr Gly Thr Gln His
 385 390 395 400
 Asp Tyr Leu Asp His Ser Asp Ile Ile Gly Trp Thr Arg Glu Gly Gly
 405 410 415
 Thr Glu Lys Pro Gly Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
 420 425 430
 Gly Gly Ser Lys Trp Met Tyr Val Gly Lys Gln His Ala Gly Lys Val
 435 440 445
 Phe Tyr Asp Leu Thr Gly Asn Arg Ser Asp Thr Val Thr Ile Asn Ser
 450 455 460
 Asp Gly Trp Gly Glu Phe Lys Val Asn Gly Gly Ser Val Ser Val Trp
 465 470 475 480
 Val Pro Arg Lys Thr Thr Val Ser Thr Ile Ala Arg Pro Ile Thr Thr
 485 490 495
 Arg Pro Trp Thr Gly Glu Phe Val Arg Trp Thr Glu Pro Arg Leu Val
 500 505 510
 Ala Trp Pro
 515

<210> 7
 <211> 1920
 <212> DNA
 <213> Bacillus licheniformis

<220>
 <221> CDS
 <222> (421)..(1872)
 <223> Termamyl

<400> 7

SEQUENCE LISTING

cggaagattg gaagtacaaa aataagcaaa agattgtcaa tcatgtcatg agccatgcgg 60
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 agattattaa aaagctgaaa gcaaaaggct atcaattggt aactgtatct cagcttgaag 180
 aagtgaagaa gcagagaggc tattgaataa atgagtagaa gcgccatata gccgcttttc 240
 ttttgaaga aaatataggg aaaatggtac ttgttaaaaaa ttcggaatat ttatacaaca 300
 tcatatgttt cacattgaaa ggggaggaga atcatgaaac aacaaaaacg gctttacgcc 360
 cgattgctga cgctgttatt tgcgctcatc ttcttgctgc ctcattctgc agcagcggcg 420
 gca aat ctt aat ggg acg ctg atg cag tat ttt gaa tgg tac atg ccc 468
 Ala Asn Leu Asn Gly Thr Leu Met Gln Tyr Phe Glu Trp Tyr Met Pro
 1 5 10 15
 aat gac ggc caa cat tgg agg cgt ttg caa aac gac tcg gca tat ttg 516
 Asn Asp Gly Gln His Trp Arg Arg Leu Gln Asn Asp Ser Ala Tyr Leu
 20 25 30
 gct gaa cac ggt att act gcc gtc tgg att ccc ccg gca tat aag gga 564
 Ala Glu His Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly
 35 40 45
 acg agc caa gcc gat gtg ggc tac ggt gct tac gac ctt tat gat tta 612
 Thr Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
 50 55 60
 ggg gag ttt cat caa aaa ggg acg gtt cgg aca aag tac ggc aca aaa 660
 Gly Glu Phe His Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys
 65 70 75 80
 gga gag ctg caa tct gcc atc aaa agt ctt cat tcc cgc gac att aac 708
 Gly Glu Leu Gln Ser Ala Ile Lys Ser Leu His Ser Arg Asp Ile Asn
 85 90 95
 gtt tac ggg gat gtg gtc atc aac cac aaa ggc ggc gct gat gcg acc 756
 Val Tyr Gly Asp Val Val Ile Asn His Lys Gly Gly Ala Asp Ala Thr
 100 105 110
 gaa gat gta acc gcc gtt gaa gtc gat ccc gct gac cgc aac cgc gta 804
 Glu Asp Val Thr Ala Val Glu Val Asp Pro Ala Asp Arg Asn Arg Val
 115 120 125
 att tca gga gaa cac cta att aaa gcc tgg aca cat ttt cat ttt ccg 852
 Ile Ser Gly Glu His Leu Ile Lys Ala Trp Thr His Phe His Phe Pro
 130 135 140
 ggg cgc ggc agc aca tac agc gat ttt aaa tgg cat tgg tac cat ttt 900
 Gly Arg Gly Ser Thr Ser Asp Phe Lys Trp His Trp Tyr His Phe
 145 150 155 160
 gac gga acc gat tgg gac gag tcc cga aag ctg aac cgc atc tat aag 948
 Asp Gly Thr Asp Trp Asp Glu Ser Arg Lys Leu Asn Arg Ile Tyr Lys
 165 170 175
 ttt caa gga aag gct tgg gat tgg gaa gtt tcc aat gaa aac gcc aac 996
 Phe Gln Gly Lys Ala Trp Asp Trp Glu Val Ser Asn Glu Asn Gly Asn
 180 185 190
 tat gat tat ttg atg tat gcc gac atc gat tat gac cat cct gat gtc 1044
 Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Tyr Asp His Pro Asp Val
 195 200 205
 gca gca gaa att aag aga tgg gcc act tgg tat gcc aat gaa ctg caa 1092
 Ala Ala Glu Ile Lys Arg Trp Gly Thr Trp Tyr Ala Asn Glu Leu Gln

SEQUENCE LISTING															
210											220				
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Leu Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys Phe Ser Phe															
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Phe Thr Val Ala Glu Tyr Trp Gln Asn Asp Leu Gly Ala Leu Glu Asn															
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tat ttg aac aaa aca aat ttt aat cat tca gtg ttt gac gtg ccg ctt															
Tyr Leu Asn Lys Thr Asn Phe Asn His Ser Val Phe Asp Val Pro Leu															
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His Tyr Gln Phe His Ala Ala Ser Thr Gln Gly Gly Gly Tyr Asp Met															
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agg aaa ttg ctg aac ggt acg gtc gtt tcc aag cat ccg ttg aaa tcg															
Arg Lys Leu Leu Asn Gly Thr Val Val Ser Lys His Pro Leu Lys Ser															
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Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu															
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Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu															
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aca agg gaa tct gga tac cct cag gtt ttc tac ggg gat atg tac ggg															
Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly															
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acg aaa gga gac tcc cag cgc gaa att cct gcc ttg aaa cac aaa att															
Thr Lys Gly Asp Ser Gln Arg Glu Ile Pro Ala Leu Lys His Lys Ile															
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gaa ccg atc tta aaa gcg aga aaa cag tat gcg tac gga gca cag cat															
Glu Pro Ile Leu Lys Ala Arg Lys Gln Tyr Ala Tyr Gly Ala Gln His															
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gat tat ttc gac cac cat gac att gtc ggc tgg aca agg gaa ggc gac															
Asp Tyr Phe Asp His His Asp Ile Val Gly Trp Thr Arg Glu Gly Asp															
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agc tcg gtt gca aat tca ggt ttg gcg gca tta ata aca gac gga ccc															
Ser Ser Val Ala Asn Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro															
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Gly Gly Ala Lys Arg Met Tyr Val Gly Arg Gln Asn Ala Gly Glu Thr															
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Trp His Asp Ile Thr Gly Asn Arg Ser Glu Pro Val Val Ile Asn Ser															
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Glu Gly Trp Gly Glu Phe His Val Asn Gly Gly Ser Val Ser Ile Tyr															
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Val Gln Arg															
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1920

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<211> 483

<212> PRT

<213> Bacillus licheniformis

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Ala Glu His Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly
 35 40 45

Thr Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu
 50 55 60

Gly Glu Phe His Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys
 65 70 75 80

Gly Glu Leu Gln Ser Ala Ile Lys Ser Leu His Ser Arg Asp Ile Asn
 85 90 95

Val Tyr Gly Asp Val Val Ile Asn His Lys Gly Gly Ala Asp Ala Thr
 100 105 110

Glu Asp Val Thr Ala Val Glu Val Asp Pro Ala Asp Arg Asn Arg Val
 115 120 125

Ile Ser Gly Glu His Leu Ile Lys Ala Trp Thr His Phe His Phe Pro
 130 135 140

Gly Arg Gly Ser Thr Tyr Ser Asp Phe Lys Trp His Trp Tyr His Phe
 145 150 155 160

Asp Gly Thr Asp Trp Asp Glu Ser Arg Lys Leu Asn Arg Ile Tyr Lys
 165 170 175

Phe Gln Gly Lys Ala Trp Asp Trp Glu Val Ser Asn Glu Asn Gly Asn
 180 185 190

Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Tyr Asp His Pro Asp Val
 195 200 205

Ala Ala Glu Ile Lys Arg Trp Gly Thr Trp Tyr Ala Asn Glu Leu Gln
 210 215 220

Leu Asp Gly Phe Arg Leu Asp Ala Val Lys His Ile Lys Phe Ser Phe
 225 230 235 240

Leu Arg Asp Trp Val Asn His Val Arg Glu Lys Thr Gly Lys Glu Met
 245 250 255

Phe Thr Val Ala Glu Tyr Trp Gln Asn Asp Leu Gly Ala Leu Glu Asn
 260 265 270

Tyr Leu Asn Lys Thr Asn Phe Asn His Ser Val Phe Asp Val Pro Leu
 275 280 285

His Tyr Gln Phe His Ala Ala Ser Thr Gln Gly Gly Gly Tyr Asp Met
 290 295 300

Arg Lys Leu Leu Asn Gly Thr Val Val Ser Lys His Pro Leu Lys Ser

SEQUENCE LISTING

	SEQUENCE LISTING																								
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Val Thr Phe Val Asp Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu																									
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Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu																									
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Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly																									
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Thr Lys Gly Asp Ser Gln Arg Glu Ile Pro Ala Leu Lys His Lys Ile																									
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Glu Pro Ile Leu Lys Ala Arg Lys Gln Tyr Ala Tyr Gly Ala Gln His																									
385						390						395								400					
Asp Tyr Phe Asp His His Asp Ile Val Gly Trp Thr Arg Glu Gly Asp																									
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Ser Ser Val Ala Asn Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro																									
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Gly Gly Ala Lys Arg Met Tyr Val Gly Arg Gln Asn Ala Gly Glu Thr																									
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Trp His Asp Ile Thr Gly Asn Arg Ser Glu Pro Val Ile Asn Ser																									
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val gln arg																									

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<213> Bacillus amyloliquefaciens
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atcagacagg gtatttttta tgctgtccag actgtccgct gtgtaaaaat aaggaataaa 180
gggggggttgt tattatttta ctgatatgta aaatataatt tgtataagaa aatgagaggg 240
agaggaaaaca tgattcaaaa acgaaagcgg acagtttcgt tcagacttgt gcttatgtgc 300
acgctgttat ttgtcagttt gccgattaca aaaacatcag cc gta aat ggc acg 354
                               Val Asn Gly Thr
                               1
ctg atg cag tat ttt gaa tgg tat acg ccg aac gac ggc cag cat tgg 402
Leu Met Gln Tyr Phe Glu Trp Tyr Thr Pro Asn Asp Gly Gln His Trp
  5                10                15                20
aaa cga ttg cag aat gat gcg gaa cat tta tcg gat atc gga atc act 450
Lys Arg Leu Gln Asn Asp Ala Glu His Leu Ser Asp Ile Gly Ile Thr
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SEQUENCE LISTING

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gga tac gga cct tat gat ttg tat gat tta gga gaa ttc cag caa aaa 546
Gly Tyr Gly Pro Tyr Asp Leu Tyr Asp Leu Gly Glu Phe Gln Gln Lys
55 60 65

ggg acg gtc aga acg aaa tac ggc aca aaa tca gag ctt caa gat gcg 594
Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys Ser Glu Leu Gln Asp Ala
70 75 80

atc ggc tca ctg cat tcc cgg aac gtc caa gta tac gga gat gtg gtt 642
Ile Gly Ser Leu His Ser Arg Asn Val Gln Val Tyr Gly Asp Val Val
85 90 95 100

ttg aat cat aag gct ggt gct gat gca aca gaa gat gta act gcc gtc 690
Leu Asn His Lys Ala Gly Ala Asp Ala Thr Glu Asp Val Thr Ala Val
105 110 115

gaa gtc aat ccg gcc aat aga aat cag gaa act tcg gag gaa tat caa 738
Glu Val Asn Pro Ala Asn Arg Asn Gln Glu Thr Ser Glu Glu Tyr Gln
120 125 130

atc aaa gcg tgg acg gat ttt cgt ttt ccg ggc cgt gga aac acg tac 786
Ile Lys Ala Trp Thr Asp Phe Arg Phe Pro Gly Arg Gly Asn Thr Tyr
135 140 145

agt gat ttt aaa tgg cat tgg tat cat ttc gac gga gcg gac tgg gat 834
Ser Asp Phe Lys Trp His Trp Tyr His Phe Asp Gly Ala Asp Trp Asp
150 155 160

gaa tcc ccg aag atc agc cgc atc ttt aag ttt cgt ggg gaa gga aaa 882
Glu Ser Arg Lys Ile Ser Arg Ile Phe Lys Phe Arg Gly Glu Gly Lys
165 170 175 180

gcg tgg gat tgg gaa gta tca agt gaa aac ggc aac tat gac tat tta 930
Ala Trp Asp Trp Glu Val Ser Ser Glu Asn Gly Asn Tyr Asp Tyr Leu
185 190 195

atg tat gct gat gtt gac tac gac cac cct gat gtc gtg gca gag aca 978
Met Tyr Ala Asp Val Asp Tyr Asp His Pro Asp Val Val Ala Glu Thr
200 205 210

aaa aaa tgg ggt atc tgg tat gcg aat gaa ctg tca tta gac ggc ttc 1026
Lys Lys Trp Gly Ile Trp Tyr Ala Asn Glu Leu Ser Leu Asp Gly Phe
215 220 225

cgt att gat gcc gcc aaa cat att aaa ttt tca ttt ctg cgt gat tgg 1074
Arg Ile Asp Ala Ala Lys His Ile Lys Phe Ser Phe Leu Arg Asp Trp
230 235 240

gtt cag gcg gtc aga cag gcg acg gga aaa gaa atg ttt acg gtt gcg 1122
Val Gln Ala Val Arg Gln Ala Thr Gly Lys Glu Met Phe Thr Val Ala
245 250 255 260

gag tat tgg cag aat aat gcc ggg aaa ctg gaa aac tac ttg aat aaa 1170
Glu Tyr Trp Gln Asn Asn Ala Gly Lys Leu Glu Asn Tyr Leu Asn Lys
265 270 275

aca agc ttt aat caa tcc gtg ttt gat gtt ccg ctt cat ttc aat tta 1218
Thr Ser Phe Asn Gln Ser Val Phe Asp Val Pro Leu His Phe Asn Leu
280 285 290

cag gcg gct tcc tca caa gga ggc gga tat gat atg agg cgt ttg ctg 1266
Gln Ala Ser Ser Gln Gly Gly Tyr Asp Met Arg Arg Leu Leu
295 300 305

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SEQUENCE LISTING

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 Glu Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu Ser Thr Val Gln
 325 330 335 340

act tgg ttt aaa ccg ctt gca tac gcc ttt att ttg aca aga gaa tcc 1410
 Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu Thr Arg Glu Ser
 345 350 355

ggt tat cct cag gtg ttc tat ggg gat atg tac ggg aca aaa ggg aca 1458
 Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly Thr Lys Gly Thr
 360 365 370

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 Ser Pro Lys Glu Ile Pro Ser Leu Lys Asp Asn Ile Glu Pro Ile Leu
 375 380 385

aaa gcg cgt aag gag tac gca tac ggg ccc cag cac gat tat att gac 1554
 Lys Ala Arg Lys Glu Tyr Ala Tyr Gly Pro Gln His Asp Tyr Ile Asp
 390 395 400

cac ccg gat gtg atc gga tgg acg agg gaa ggt gac agc tcc gcc gcc 1602
 His Pro Asp Val Ile Gly Trp Thr Arg Glu Gly Asp Ser Ser Ala Ala
 405 410 415 420

aaa tca ggt ttg gcc gct tta atc acg gac gga ccc ggc gga tca aag 1650
 Lys Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro Gly Gly Ser Lys
 425 430 435

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 Arg Met Tyr Ala Gly Leu Lys Asn Ala Gly Glu Thr Trp Tyr Asp Ile
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 Thr Gly Asn Arg Ser Asp Thr Val Lys Ile Gly Ser Asp Gly Trp Gly
 455 460 465

gag ttt cat gta aac gat ggg tcc gtc tcc att tat gtt cag aaa taa 1794
 Glu Phe His Val Asn Asp Gly Ser Val Ser Ile Tyr Val Gln Lys
 470 475 480

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gtgacaaatc cgggtttttgc gccgttttggc tttttcacat gtctgatttt tgtataatca 1974

acaggcacgg agccggaatc tttcgccttg gaaaaataag cggcgatcgt agctgcttcc 2034

aatatggatt gttcatcggg atcgctgctt ttaatcacaa cgtgggatcc 2084

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 <212> PRT
 <213> Bacillus amyloliquefaciens

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 Gly Gln His Trp Lys Arg Leu Gln Asn Asp Ala Glu His Leu Ser Asp
 20 25 30

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Ile Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly Leu Ser
 35 40 45
 Gln Ser Asp Asn Gly Tyr Gly Pro Tyr Asp Leu Tyr Asp Leu Gly Glu
 50 55 60
 Phe Gln Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Lys Ser Glu
 65 70 75 80
 Leu Gln Asp Ala Ile Gly Ser Leu His Ser Arg Asn Val Gln Val Tyr
 85 90 95
 Gly Asp Val Val Leu Asn His Lys Ala Gly Ala Asp Ala Thr Glu Asp
 100 105 110
 Val Thr Ala Val Glu Val Asn Pro Ala Asn Arg Asn Gln Glu Thr Ser
 115 120 125
 Glu Glu Tyr Gln Ile Lys Ala Trp Thr Asp Phe Arg Phe Pro Gly Arg
 130 135 140
 Gly Asn Thr Tyr Ser Asp Phe Lys Trp His Trp Tyr His Phe Asp Gly
 145 150 155 160
 Ala Asp Trp Asp Glu Ser Arg Lys Ile Ser Arg Ile Phe Lys Phe Arg
 165 170 175
 Gly Glu Gly Lys Ala Trp Asp Trp Glu Val Ser Ser Glu Asn Gly Asn
 180 185 190
 Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Tyr Asp His Pro Asp Val
 195 200 205
 Val Ala Glu Thr Lys Lys Trp Gly Ile Trp Tyr Ala Asn Glu Leu Ser
 210 215 220
 Leu Asp Gly Phe Arg Ile Asp Ala Ala Lys His Ile Lys Phe Ser Phe
 225 230 235 240
 Leu Arg Asp Trp Val Gln Ala Val Arg Gln Ala Thr Gly Lys Glu Met
 245 250 255
 Phe Thr Val Ala Glu Tyr Trp Gln Asn Asn Ala Gly Lys Leu Glu Asn
 260 265 270
 Tyr Leu Asn Lys Thr Ser Phe Asn Gln Ser Val Phe Asp Val Pro Leu
 275 280 285
 His Phe Asn Leu Gln Ala Ala Ser Ser Gln Gly Gly Gly Tyr Asp Met
 290 295 300
 Arg Arg Leu Leu Asp Gly Thr Val Val Ser Arg His Pro Glu Lys Ala
 305 310 315 320
 Val Thr Phe Val Glu Asn His Asp Thr Gln Pro Gly Gln Ser Leu Glu
 325 330 335
 Ser Thr Val Gln Thr Trp Phe Lys Pro Leu Ala Tyr Ala Phe Ile Leu
 340 345 350
 Thr Arg Glu Ser Gly Tyr Pro Gln Val Phe Tyr Gly Asp Met Tyr Gly
 355 360 365
 Thr Lys Gly Thr Ser Pro Lys Glu Ile Pro Ser Leu Lys Asp Asn Ile
 370 375 380
 Glu Pro Ile Leu Lys Ala Arg Lys Glu Tyr Ala Tyr Gly Pro Gln His
 385 390 395 400

SEQUENCE LISTING

Asp Tyr Ile Asp His Pro Asp Val Ile Gly Trp Thr Arg Glu Gly Asp
 405 410 415
 Ser Ser Ala Ala Lys Ser Gly Leu Ala Ala Leu Ile Thr Asp Gly Pro
 420 425 430
 Gly Gly Ser Lys Arg Met Tyr Ala Gly Leu Lys Asn Ala Gly Glu Thr
 435 440 445
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 <212> DNA
 <213> Bacillus sp.

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 <222> (1)..(1458)
 <223> AA560

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 aac cta aaa gat aaa ggg atc tca gcg gtt tgg att cct cct gca tgg 144
 Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp
 35 40 45
 aag ggt gcc tct caa aat gat gtg ggg tat ggt gct tat gat ctg tat 192
 Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
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 gat tta gga gaa ttc aat caa aaa gga acc att cgt aca aaa tat gga 240
 Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly
 65 70 75 80
 acg cgc aat cag tta caa gct gca gtt aac gcc ttg aaa agt aat gga 288
 Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly
 85 90 95
 att caa gtg tat ggc gat gtt gta atg aat cat aaa ggg gga gca gac 336
 Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
 100 105 110
 gct acc gaa atg gtt agg gca gtt gaa gta aac ccg aat aat aga aat 384
 Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
 115 120 125
 caa gaa gtg tcc ggt gaa tat aca att gag gct tgg aca aag ttt gac 432
 Gln Glu Val Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
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SEQUENCE LISTING

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Ile	Tyr	Lys	Phe 180	Arg	Gly	Asp	Gly	Lys 185	Gly	Trp	Asp	Trp	Glu	Val	Asp	
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Thr	Glu	Asn 195	Gly	Asn	Tyr	Asp	Tyr 200	Leu	Met	Tyr	Ala	Asp 205	Ile	Asp	Met	
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Ile	Lys	Tyr	Ser 245	Phe	Thr	Arg	Asp	Trp	Ile 250	Asn	His	Val	Arg	Ser	Ala 255	
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Gly	Ala	Ile 275	Glu	Asn	Tyr	Leu	Asn 280	Lys	Thr	Asn	Trp	Asn 285	His	Ser	Val	
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Gly	Asn	Tyr	Asp	Met	Arg 310	Gln	Ile	Phe	Asn 315	Gly	Thr	Val	Val	Gln	Arg 320	
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His	Pro	Met	His 325	Ala	Val	Thr	Phe	Val	Asp 330	Asn	His	Asp	Ser	Gln 335	Pro	
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Tyr	Ala	Leu 355	Thr	Leu	Thr	Arg	Glu 360	Gln	Gly	Tyr	Pro	Ser 365	Val	Phe	Tyr	
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Gly	Asp	Tyr	Tyr	Gly 370	Ile	Pro 375	Thr	His	Gly	Val 380	Pro	Ala	Met	Lys	Ser	
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Lys	Ile	Asp	Pro	Ile 390	Leu	Glu	Ala	Arg	Gln 395	Lys	Tyr	Ala	Tyr	Gly	Arg 400	
caa	aat	gac	tac	tta	gac	cat	cat	aat	atc	atc	ggt	tgg	aca	cgt	gaa	1248
Gln	Asn	Asp	Tyr 405	Leu	Asp	His	His	Asn	Ile 410	Ile	Gly	Trp	Thr	Arg 415	Glu	
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SEQUENCE LISTING

Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
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 Gly Ala Gly Gly Asn Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
 435 440 445

caa gtt tgg acc gat atc act gga aat cgt gca ggt act gtt acg att 1392
 Gln Val Trp Thr Asp Ile Thr Gly Asn Arg Ala Gly Thr Val Thr Ile
 450 455 460

aat gct gat gga tgg ggt aat ttt tct gta aat gga gga tca gtt tct 1440
 Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480

att tgg gta aac aaa taa 1458
 Ile Trp Val Asn Lys
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<210> 12
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 <212> PRT
 <213> Bacillus sp.

<400> 12

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 20 25 30

Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp
 35 40 45

Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
 50 55 60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly
 65 70 75 80

Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly
 85 90 95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
 100 105 110

Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
 115 120 125

Gln Glu Val Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
 130 135 140

Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr
 145 150 155 160

His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg
 165 170 175

Ile Tyr Lys Phe Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp
 180 185 190

Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met
 195 200 205

Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr
 210 215 220

SEQUENCE LISTING

Thr Asn Thr Leu Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
 225 230 235 240
 Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala
 245 250 255
 Thr Gly Lys Asn Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
 260 265 270
 Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
 275 280 285
 Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly
 290 295 300
 Gly Asn Tyr Asp Met Arg Gln Ile Phe Asn Gly Thr Val Val Gln Arg
 305 310 315 320
 His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
 325 330 335
 Glu Glu Ala Leu Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala
 340 345 350
 Tyr Ala Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
 355 360 365
 Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
 370 375 380
 Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Arg
 385 390 395 400
 Gln Asn Asp Tyr Leu Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
 405 410 415
 Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430
 Gly Ala Gly Gly Asn Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
 435 440 445
 Gln Val Trp Thr Asp Ile Thr Gly Asn Arg Ala Gly Thr Val Thr Ile
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 Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480
 Ile Trp Val Asn Lys
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<210> 13

<211> 485

<212> PRT

<213> Bacillus sp. 707

<400> 13

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 20 25 30
 Asn Leu Lys Ser Lys Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Trp
 35 40 45
 Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr

SEQUENCE LISTING

50	55	60
Asp 65	Leu Gly Glu Phe Asn 70	Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly 80
Thr Arg Ser Gln 85	Leu Gln Ala Ala Val 90	Thr Ser Leu Lys Asn Asn Gly 95
Ile Gln Val 100	Tyr Gly Asp Val Val 105	Met Asn His Lys Gly Gly Ala Asp 110
Ala Thr Glu Met Val 115	Arg Ala Val Glu Val 120	Asn Pro Asn Asn Arg Asn 125
Gln Glu Val Thr Gly Glu 130	Tyr Thr Ile Glu Ala Trp 135	Thr Arg Phe Asp 140
Phe Pro Gly Arg Gly 145	Asn Thr His Ser Ser 150	Phe Lys Trp Arg Trp Tyr 155 160
His Phe Asp Gly Val 165	Asp Trp Asp Gln Ser 170	Arg Arg Leu Asn Asn Arg 175
Ile Tyr Lys Phe Arg Gly His 180	Gly Lys Ala Trp Asp Trp 185	Glu Val Asp 190
Thr Glu Asn Gly Asn Tyr 195	Asp Tyr Leu Met Tyr Ala 200	Asp Ile Asp Met 205
Asp His Pro Glu Val Val 210	Asn Glu Leu Arg Asn 215	Trp Gly Val Trp Tyr 220
Thr Asn Thr Leu Gly 225	Leu Asp Gly Phe Arg 230	Ile Asp Ala Val Lys His 235 240
Ile Lys Tyr Ser Phe Thr Arg Asp 245	Trp Ile Asn His Val Arg 250	Ser Ala 255
Thr Gly Lys Asn Met Phe Ala Val 260	Ala Glu Phe Trp Lys Asn Asp 265	Leu 270
Gly Ala Ile Glu Asn Tyr Leu 275	Gln Lys Thr Asn Trp Asn His Ser Val 280	285
Phe Asp Val Pro Leu His Tyr 290	Asn Leu Tyr Asn Ala Ser Lys Ser Gly 295	300
Gly Asn Tyr Asp Met Arg Asn 305	Ile Phe Asn Gly Thr Val Val Gln Arg 310	315 320
His Pro Ser His Ala Val Thr Phe Val 325	Asp Asn His Asp Ser Gln Pro 330	335
Glu Glu Ala Leu Glu Ser Phe Val 340	Glu Glu Trp Phe Lys Pro Leu Ala 345	350
Tyr Ala Leu Thr Leu Thr Arg 355	Glu Gln Gly Tyr Pro Ser Val Phe Tyr 360	365
Gly Asp Tyr Tyr Gly Ile Pro 370	Thr His Gly Val Pro Ala Met Arg Ser 375	380
Lys Ile Asp Pro Ile Leu Glu Ala Arg 385	Gln Lys Tyr Ala Tyr Gly Lys 390	395 400
Gln Asn Asp Tyr Leu Asp His His Asn 405	Ile Ile Gly Trp Thr Arg Glu 410	415

SEQUENCE LISTING

Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430

Gly Ala Gly Gly Ser Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
 435 440 445

Gln Val Trp Ser Asp Ile Thr Gly Asn Arg Thr Gly Thr Val Thr Ile
 450 455 460

Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480

Ile Trp Val Asn Lys
 485

<210> 14
 <211> 27
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 <213> Artificial Sequence
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 <223> Primer 22149
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 24

<210> 15
 <211> 29
 <212> DNA
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 <223> Primer 24814
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 GATCACCCGC GATACCGTC
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<210> 16
 <211> 31
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 <213> Artificial Sequence
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 <223> Primer #24
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 GAATGTATGT CGGCCGGCAA AACGCCGGTG A
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<210> 17
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 <212> DNA
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 <223> Primer #27
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<210> 18
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 <223> Primer #312
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48

<210> 19
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SEQUENCE LISTING

<223> Primer #290
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<210> 20
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 <223> Primer #314
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<210> 22
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 <223> Primer #296
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<210> 23
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 <212> DNA
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 <223> Primer #305
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<210> 24
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ttg atg aaa aga tgg gta gta gca atg ctg gca gtg tta ttt tta ttt 109
 Met Lys Arg Trp Val Val Ala Met Leu Ala Val Leu Phe Leu Phe
 -20 -15 -10

cct tgg gta gta gtt gca gat ggc ttg aat gga acg atg atg cag tat 157
 Pro Ser Val Val Val Ala Asp Gly Leu Asn Gly Thr Met Met Gln Tyr
 -5 -1 1 5 10

tat gag tgg cat cta gag aat gat ggg caa cac tgg aat cgg ttg cat 205

SEQUENCE LISTING

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Asp	Asp	Ala	Glu	Ala	Leu	Ser	Asn	Ala	Gly	Ile	Thr	Ala	Ile	Trp	Ile	
		30						35				40				
ccc	cca	gcc	tac	aaa	gga	aat	agt	cag	gct	gat	gtt	ggg	tat	ggt	gca	301
Pro	Pro	Ala	Tyr	Lys	Gly	Asn	Ser	Gln	Ala	Asp	Val	Gly	Tyr	Gly	Ala	
		45					50					55				
tac	gac	ctt	tat	gat	tta	ggg	gag	ttt	aat	caa	aaa	ggt	acc	gtt	cga	349
Tyr	Asp	Leu	Tyr	Asp	Leu	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Val	Arg	
	60					65					70					
acg	aaa	tac	ggg	aca	aag	gct	cag	ctt	gag	cga	gct	ata	ggg	tcc	cta	397
Thr	Lys	Tyr	Gly	Thr	Lys	Ala	Gln	Leu	Glu	Arg	Ala	Ile	Gly	Ser	Leu	
	75				80					85					90	
aag	tcg	aat	gat	atc	aat	gtt	tat	ggg	gat	gtc	gta	atg	aat	cat	aaa	445
Lys	Ser	Asn	Asp	Ile	Asn	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	
				95					100					105		
tta	gga	gct	gat	ttc	acg	gag	gca	gtg	caa	gct	gtt	caa	gta	aat	cct	493
Leu	Gly	Ala	Asp	Phe	Thr	Glu	Ala	Val	Gln	Ala	Val	Gln	Val	Asn	Pro	
			110					115					120			
tcg	aac	cgt	tgg	cag	gat	att	tca	ggt	gtc	tac	acg	att	gat	gca	tgg	541
Ser	Asn	Arg	Trp	Gln	Asp	Ile	Ser	Gly	Val	Tyr	Thr	Ile	Asp	Ala	Trp	
		125					130					135				
acg	gga	ttt	gac	ttt	cca	ggg	cgc	aac	aat	gcc	tat	tcc	gat	ttt	aaa	589
Thr	Gly	Phe	Asp	Phe	Pro	Gly	Arg	Asn	Asn	Ala	Tyr	Ser	Asp	Phe	Lys	
	140					145					150					
tgg	aga	tgg	ttc	cat	ttt	aat	ggc	gtt	gac	tgg	gat	caa	cgc	tat	caa	637
Trp	Arg	Trp	Phe	His	Phe	Asn	Gly	Val	Asp	Trp	Asp	Gln	Arg	Tyr	Gln	
	155				160					165					170	
gaa	aac	cat	ctt	ttt	cgc	ttt	gca	aat	acg	aac	tgg	aac	tgg	cga	gtg	685
Glu	Asn	His	Leu	Phe	Arg	Phe	Ala	Asn	Thr	Asn	Trp	Asn	Trp	Arg	Val	
				175					180					185		
gat	gaa	gag	aat	ggt	aat	tat	gac	tat	tta	tta	gga	tcg	aac	att	gac	733
Asp	Glu	Glu	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Leu	Gly	Ser	Asn	Ile	Asp	
			190					195					200			
ttt	agc	cac	cca	gag	gtt	caa	gag	gaa	tta	aag	gat	tgg	ggg	agc	tgg	781
Phe	Ser	His	Pro	Glu	Val	Gln	Glu	Glu	Leu	Lys	Asp	Trp	Gly	Ser	Trp	
		205				210						215				
ttt	acg	gat	gag	cta	gat	tta	gat	ggg	tat	cga	ttg	gat	gct	att	aag	829
Phe	Thr	Asp	Glu	Leu	Asp	Leu	Asp	Gly	Tyr	Arg	Leu	Asp	Ala	Ile	Lys	
	220					225					230					
cat	att	cca	ttc	tgg	tat	acg	tca	gat	tgg	gtt	agg	cat	cag	cga	agt	877
His	Ile	Pro	Phe	Trp	Tyr	Thr	Ser	Asp	Trp	Val	Arg	His	Gln	Arg	Ser	
	235				240					245					250	
gaa	gca	gac	caa	gat	tta	ttt	gtc	gta	ggg	gag	tat	tgg	aag	gat	gac	925
Glu	Ala	Asp	Gln	Asp	Leu	Phe	Val	Val	Gly	Glu	Tyr	Trp	Lys	Asp	Asp	
				255					260					265		
gta	ggt	gct	ctc	gaa	ttt	tat	tta	gat	gaa	atg	aat	tgg	gag	atg	tct	973
Val	Gly	Ala	Leu	Glu	Phe	Tyr	Leu	Asp	Glu	Met	Asn	Trp	Glu	Met	Ser	
			270					275					280			
cta	ttc	gat	gtt	ccg	ctc	aat	tat	aat	ttt	tac	cgg	gct	tca	aag	caa	1021

SEQUENCE LISTING

Leu Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Lys Gln
 285 290 295
 ggc gga agc tat gat atg cgt aat att tta cga gga tct tta gta gaa 1069
 Gly Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val Glu
 300 305 310
 gca cat ccg att cat gca gtt acg ttt gtt gat aat cat gat act cag 1117
 Ala His Pro Ile His Ala Val Thr Phe Val Asp Asn His Asp Thr Gln
 315 320 325 330
 cca gga gag tca tta gaa tca tgg gtc gct gat tgg ttt aag cca ctt 1165
 Pro Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro Leu
 335 340 345
 gct tat gcg aca atc ttg acg cgt gaa ggt ggt tat cca aat gta ttt 1213
 Ala Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe
 350 355 360
 tac ggt gac tac tat ggg att cct aac gat aac att tca gct aag aag 1261
 Tyr Gly Asp Tyr Tyr Gly Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys
 365 370 375
 gat atg att gat gag ttg ctt gat gca cgt caa aat tac gca tat ggc 1309
 Asp Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly
 380 385 390
 aca caa cat gac tat ttt gat cat tgg gat atc gtt gga tgg aca aga 1357
 Thr Gln His Asp Tyr Phe Asp His Trp Asp Ile Val Gly Trp Thr Arg
 395 400 405 410
 gaa ggt aca tcc tca cgt cct aat tcg ggt ctt gct act att atg tcc 1405
 Glu Gly Thr Ser Ser Arg Pro Asn Ser Gly Leu Ala Thr Ile Met Ser
 415 420 425
 aat ggt cct gga gga tca aaa tgg atg tac gta gga cag caa cat gca 1453
 Asn Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Gln Gln His Ala
 430 435 440
 gga caa acg tgg aca gat tta act ggc aat cac gcg gcg tcg gtt acg 1501
 Gly Gln Thr Trp Thr Asp Leu Thr Gly Asn His Ala Ala Ser Val Thr
 445 450 455
 att aat ggt gat ggc tgg ggc gaa ttc ttt aca aat gga gga tct gta 1549
 Ile Asn Gly Asp Gly Trp Gly Glu Phe Phe Thr Asn Gly Gly Ser Val
 460 465 470
 tcc gtg tat gtg aac caa taataaaaag ccttgagaag ggattcctcc 1597
 Ser Val Tyr Val Asn Gln
 475 480
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 Glu Trp His Leu Glu Asn Asp Gly Gln His Trp Asn Arg Leu His Asp
 15 20 25

SEQUENCE LISTING

Asp Ala Glu Ala Leu Ser Asn Ala Gly Ile Thr Ala Ile Trp Ile Pro
 30 35 40

Pro Ala Tyr Lys Gly Asn Ser Gln Ala Asp Val Gly Tyr Gly Ala Tyr
 45 50 55

Asp Leu Tyr Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val Arg Thr
 60 65 70 75

Lys Tyr Gly Thr Lys Ala Gln Leu Glu Arg Ala Ile Gly Ser Leu Lys
 80 85 90

Ser Asn Asp Ile Asn Val Tyr Gly Asp Val Val Met Asn His Lys Leu
 95 100 105

Gly Ala Asp Phe Thr Glu Ala Val Gln Ala Val Gln Val Asn Pro Ser
 110 115 120

Asn Arg Trp Gln Asp Ile Ser Gly Val Tyr Thr Ile Asp Ala Trp Thr
 125 130 135

Gly Phe Asp Phe Pro Gly Arg Asn Asn Ala Tyr Ser Asp Phe Lys Trp
 140 145 150 155

Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu
 160 165 170

Asn His Leu Phe Arg Phe Ala Asn Thr Asn Trp Asn Trp Arg Val Asp
 175 180 185

Glu Glu Asn Gly Asn Tyr Asp Tyr Leu Leu Gly Ser Asn Ile Asp Phe
 190 195 200

Ser His Pro Glu Val Gln Glu Glu Leu Lys Asp Trp Gly Ser Trp Phe
 205 210 215

Thr Asp Glu Leu Asp Leu Asp Gly Tyr Arg Leu Asp Ala Ile Lys His
 220 225 230 235

Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg Ser Glu
 240 245 250

Ala Asp Gln Asp Leu Phe Val Val Gly Glu Tyr Trp Lys Asp Asp Val
 255 260 265

Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu
 270 275 280

Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Lys Gln Gly
 285 290 295

SEQUENCE LISTING

Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala
 300 305 310 315
 His Pro Ile His Ala Val Thr Phe Val Asp Asn His Asp Thr Gln Pro
 320 325 330
 Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro Leu Ala
 335 340 345
 Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr
 350 355 360
 Gly Asp Tyr Tyr Gly Ile Pro Asn Asp Asn Ile Ser Ala Lys Lys Asp
 365 370 375
 Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr
 380 385 390 395
 Gln His Asp Tyr Phe Asp His Trp Asp Ile Val Gly Trp Thr Arg Glu
 400 405 410
 Gly Thr Ser Ser Arg Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asn
 415 420 425
 Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Gln Gln His Ala Gly
 430 435 440
 Gln Thr Trp Thr Asp Leu Thr Gly Asn His Ala Ala Ser Val Thr Ile
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actagcactc ttcagggact aaaccacctt ttttccaaaa atgacatcat ataaacaaat 120

SEQUENCE LISTING

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 aggtatttg atg aga aga tgg gta gta gca atg ttg gca gtg tta ttt tta 231
 Met Arg Arg Trp Val Val Ala Met Leu Ala Val Leu Phe Leu
 -20 -15 -10
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 Phe Pro Ser Val Val Val Ala Asp Gly Leu Asn Gly Thr Met Met Gln
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 Tyr Tyr Glu Trp His Leu Glu Asn Asp Gly Gln His Trp Asn Arg Leu
 10 15 20 25
 cac gat gat gcc gca gct ttg agt gat gct ggt att aca gct att tgg 375
 His Asp Asp Ala Ala Ala Leu Ser Asp Ala Gly Ile Thr Ala Ile Trp
 30 35 40
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 Ile Pro Pro Ala Tyr Lys Gly Asn Ser Gln Ala Asp Val Gly Tyr Gly
 45 50 55
 gca tac gat ctt tat gat tta gga gag ttc aat caa aag ggt act gtt 471
 Ala Tyr Asp Leu Tyr Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Val
 60 65 70
 cga acg aaa tac gga act aag gca cag ctt gaa cga gct att ggg tcc 519
 Arg Thr Lys Tyr Gly Thr Lys Ala Gln Leu Glu Arg Ala Ile Gly Ser
 75 80 85
 ctt aaa tct aat gat atc aat gta tac gga gat gtc gtg atg aat cat 567
 Leu Lys Ser Asn Asp Ile Asn Val Tyr Gly Asp Val Val Met Asn His
 90 95 100 105
 aaa atg gga gct gat ttt acg gag gca gtg caa gct gtt caa gta aat 615
 Lys Met Gly Ala Asp Phe Thr Glu Ala Val Gln Ala Val Gln Val Asn
 110 115 120
 cca acg aat cgt tgg cag gat att tca ggt gcc tac acg att gat gcg 663
 Pro Thr Asn Arg Trp Gln Asp Ile Ser Gly Ala Tyr Thr Ile Asp Ala
 125 130 135
 tgg acg ggt ttc gac ttt tca ggg cgt aac aac gcc tat tca gat ttt 711
 Trp Thr Gly Phe Asp Phe Ser Gly Arg Asn Asn Ala Tyr Ser Asp Phe
 140 145 150
 aag tgg aga tgg ttc cat ttt aat ggt gtt gac tgg gat cag cgc tat 759
 Lys Trp Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr
 155 160 165
 caa gaa aat cat att ttc cgc ttt gca aat acg aac tgg aac tgg cga 807
 Gln Glu Asn His Ile Phe Arg Phe Ala Asn Thr Asn Trp Asn Trp Arg
 170 175 180 185
 gtg gat gaa gag aac ggt aat tat gat tac ctg tta gga tcg aat atc 855
 Val Asp Glu Glu Asn Gly Asn Tyr Asp Tyr Leu Leu Gly Ser Asn Ile
 190 195 200
 gac ttt agt cat cca gaa gta caa gat gag ttg aag gat tgg ggt agc 903
 Asp Phe Ser His Pro Glu Val Gln Asp Glu Leu Lys Asp Trp Gly Ser
 205 210 215
 tgg ttt acc gat gag tta gat ttg gat ggt tat cgt tta gat gct att 951
 Trp Phe Thr Asp Glu Leu Asp Leu Asp Gly Tyr Arg Leu Asp Ala Ile
 220 225 230
 aaa cat att cca ttc tgg tat aca tct gat tgg gtt cgg cat cag cgc 999
 Lys His Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg

SEQUENCE LISTING

235	240	245	
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gac gta ggt gct ctc gaa ttt tat tta gat gaa atg aat tgg gag atg Asp Val Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met 270 275 280			1095
tct cta ttc gat gtt cca ctt aat tat aat ttt tac cgg gct tca caa Ser Leu Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Gln 285 290 295			1143
caa ggt gga agc tat gat atg cgt aat att tta cga gga tct tta gta Gln Gly Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val 300 305 310			1191
gaa gcg cat ccg atg cat gca gtt acg ttt gtt gat aat cat gat act Glu Ala His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Thr 315 320 325			1239
cag cca ggg gag tca tta gag tca tgg gtt gct gat tgg ttt aag cca Gln Pro Gly Glu Ser Leu Glu Ser Trp Val Ala Asp Trp Phe Lys Pro 330 335 340 345			1287
ctt gct tat gcg aca att ttg acg cgt gaa ggt ggt tat cca aat gta Leu Ala Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val 350 355 360			1335
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aaa gat atg att gat gag ctg ctt gat gca cgt caa aat tac gca tat Lys Asp Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr 380 385 390			1431
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140 145 150 155Arg Trp Phe His Phe Asn Gly Val Asp Trp Asp Gln Arg Tyr Gln Glu
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220 225 230 235Ile Pro Phe Trp Tyr Thr Ser Asp Trp Val Arg His Gln Arg Asn Glu
240 245 250

SEQUENCE LISTING

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Gly Ala Leu Glu Phe Tyr Leu Asp Glu Met Asn Trp Glu Met Ser Leu
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Phe Asp Val Pro Leu Asn Tyr Asn Phe Tyr Arg Ala Ser Gln Gln Gly
 285 290 295

Gly Ser Tyr Asp Met Arg Asn Ile Leu Arg Gly Ser Leu Val Glu Ala
 300 305 310 315

His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Thr Gln Pro
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Tyr Ala Thr Ile Leu Thr Arg Glu Gly Gly Tyr Pro Asn Val Phe Tyr
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Met Ile Asp Glu Leu Leu Asp Ala Arg Gln Asn Tyr Ala Tyr Gly Thr
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Gln His Asp Tyr Phe Asp His Trp Asp Val Val Gly Trp Thr Arg Glu
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Gly Pro Gly Gly Ser Lys Trp Met Tyr Val Gly Arg Gln Asn Ala Gly
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Gln Thr Trp Thr Asp Leu Thr Gly Asn Asn Gly Ala Ser Val Thr Ile
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 Ala Glu His Gly Ile Thr Ala Val Trp Ile Pro Pro Ala Tyr Lys Gly
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 Val Tyr Gly Asp Val Val Ile Asn His Lys Gly Gly Ala Asp Ala Thr
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 Ile Ser Gly Glu His Leu Ile Lys Ala Trp Thr His Phe His Phe Pro
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SEQUENCE LISTING

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SEQUENCE LISTING

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1920